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GENETIC VARIABILITY AND BREEDING POTENTIAL OF BARLEY (*Hordeum vulgare* L) LANDRACES FROM NORTH SHEWA IN ETHIOPIA

By

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DECLARATION

I declare that the thesis hereby submitted by me for the Philosophiae Doctor degree at the University of Free State is my own independent work and has not previously been submitted by me at another university/faculty. I furthermore cede copyright of the thesis in favour of the University of Free State.

Signed on 20th of May, 2003 at the University of Free State, Bloemfontein, South Africa.

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DEDICATION

Dedicated to my father Assefa Gebeyaneh and my mother Lakech Haile Mariam

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ABBREVIATIONS AND SYMBOLS

$\overline{H'}$	= mean diversity index
%	= percent
µl	= micro liter
AFLP	= amplified fragment length polymorphism
APS	= ammonium persulfate
BM	= biomass
Cov	= covariance
DHE	= days to heading
DMA	= days to maturity
DNA	= deoxyribonucleic acid
GA	= genetic advance
GCA	= general combining ability
GCV	= genotypic coefficient of variance
GD	= genetic distance
GY/SP	= grain yield per spike
H'	= diversity index
h^2b	= heritability (broad sense)
h^2n	= hertitability (narrow sense)
HI	= harvest index
HPH	= high-parent heterosis
IAR	= Institute of Agricultural Research
IBPGR	= International Board for Plant Genetic Resources
ICARDA	= International Center for Agricultural Research Center in the Dry Areas
mA	= milliamper
mg	= milligram
ml	= milliliter
MPH	= mid-parent heterosis
NCSS	= number cruncher statistical system

ng	= nanogram
NS/SP	= number of seeds per spike
°C	= degree Celsius
PC	= principal component
PCR	= polymerase chain reaction
PCV	= phenotypic coefficient of variance
PLH	= plant height
ppm	= parts per million
RAPD	= random amplified polymorphic DNA
RFLP	= restriction fragment length polymorphism
rpm	= rotation per minute
SCA	= specific combining ability
SDS-PAGE	= sodium dodecyl sulfate polyacrylamide gel electrophoresis
SE	= standard error
SPL	= spike length
SSR	= simple sequence repeat
TEMED	= N,N,N^1,N^1-tetramethyleethylenediamine
UPGMA	= unweighted pair group method using arithmetic averages
δ^2	= variance
δ^2_A	= additive variance
δ^2_D	= dominance variance
$\delta^2 g$	= genotypic variance
δ^2_{gca}	= variance of general combining ability
$\delta^2 p$	= phenotypic variance
δ^2_{sca}	= variance of specific combining ability
χ^2	= chi-square
SCA	= specific combining ability

CHAPTER I

GENERAL INTRODUCTION

It is thought that man went through a three-phase development. He was first a hunter, then a herder, and then became a cultivator (Harlan, 1992). Having become a herder, it was not difficult to pass to the next phase after realising that seeds can be sown to produce plants when and where desired. There are different concepts and views as to how man began agriculture (agriculture as divine gift, domestication for religious reasons, agriculture as discovery, agriculture by stress, and agriculture as an extension of gathering). Whichever concept or view is considered, barley is believed to be one of the earliest crops domesticated in the Near East, and the present distribution of wild barley together with archaeological evidence point to a rather specific part of the region. However, variation is not notable in the centre of origin, and most of the races of barley occur elsewhere (Harlan, 1992). Barley has become, perhaps, the most widely grown of major crops, being cultivated from above the Arctic Circle to southern Argentina and Chile as well as the tropics. Each geographical and ecological region has its own set of cultivars with characteristic concentrations of particular genetic traits. The Ethiopian plateau, for example, is especially favourable for the development of leaf diseases and barley has responded over the centuries by developing high frequencies of genes for resistance. Genes conditioning irregular (seeds formed in some of the lateral spikelets) and deficient (lateral spikelets suppressed) head types are also common in Ethiopian barleys.

Traditionally, field crops consisted of landrace populations that have evolved through centuries of natural and human selection and are still grown whenever traditional agriculture is practiced. Landrace populations are highly variable in appearance, but they are each identifiable and usually have local names. In the central highlands of Ethiopia, for instance, landraces or farmers cultivars recognized by different vernacular names are widely grown at different localities and environments. Some are early maturing and some late. The diversity of the landraces provides security to the farmers against adverse growing conditions and is crucial for obtaining sustained yield. Because of its early maturity or drought tolerance, barley serves to relieve food shortage that occasionally occurs in the highlands during the long rainy season. It is a crop well

adapted to less fertile land than wheat and is grown in different production systems (for example the long cycle, *meher* barley, the short season, *belg* barley, residual and early barley production systems). The yield potential of barley landraces was also recognized in the past when the landraces used as checks in national variety trials conducted with no fertilizer were found to be superior in grain yield to the improved, fertilizer responsive barley varieties.

The crop is produced annually on an area of nearly 0.9 million hectares and the annual production is estimated to be 1 million tons (Lakew et al., 1996). Despite its importance and long history of cultivation, the average national productivity is quite low, 1.1 t/ha (Lakew et al., 1996). Seasonal waterlogging is among the major factors limiting barley production in the central highlands because the soils are either vertisol which have a high content of montimorilinic clay (60-70 %) or because of relatively level topography (slopes generally less than 8 %) (Debele, 1995). Much of the waterlogging problem on barley, especially in the central highlands, arises from excess and continuous rainfall in the months of July and August causing a total crop failure if the excess water in the field is not properly drained. Farmers in the central highlands have recognized the adverse effects of waterlogging on barley production; however, they have hardly any effective modern technology that can be used to drain the excess surface water from their fields. Most of the farmers use the traditional drainage furrow made during planting at about 2 to 3 m intervals and maintained during the season. Surface drainage using camber beds, open trenches, broad beds and furrows has drastically increased grain yields. However, economic viability of effective drainage systems for the small-scale farmers is questionable. Soil burning, *guie*, is a common practice on flat lands where waterlogging is a critical problem and it is well recognized by farmers that *guie* improves soil fertility and drainage. The yield of barley following *guie* is high in the first crop season and declines rapidly in the second and third seasons and the land is left fallow for five to seven years. Each landrace has a reputation for adaptation to particular soil types, heavy clay soils with poor drainage or well-drained light soils. Hence, as part of their strategy to cope with the drainage problem, farmers growing barley in areas prone to waterlogging have recognized differences among landraces in tolerance to waterlogging and use those adapted to the conditions. Moreover, the variability between cultivars allowed farmers to shift between cultivars for planting depending on environmental circumstances. Farmers have identified, for example, early maturing barley types that fit

the short rainy season and reach maturity towards the onset of the main rainy season. These types of barley are also planted in situations when the onset of the main season is delayed and planting of the long cycle barley is impossible.

Ethiopian barley germplasm has been used internationally as sources of useful genes for traits such as disease resistance (Qualset, 1975) and protein quality (Munck et al., 1970; Asfaw, 1989). By contrast, within Ethiopia, the locally adapted barley germplasm remains unexploited (Lakew et al., 1997). One of the reasons why the indigenous barley germplasm, especially the commonly grown farmers' cultivars have not been effectively utilized in the national barley breeding programme is that the materials have not been adequately evaluated (Asfaw, 1989c). In other words, the diversity and genetic worth of these materials was not properly understood. Various researchers have analyzed the phenotypic diversity (Demissie & Bjornstad, 1996; Negasa, 1985; Asfaw, 1988) and isozyme variation (Bekele, 1983b; 1983c; Demissie et al., 1997) of Ethiopian barley landraces but all were done on random germplasm collections or accessions based on samples from various parts of Ethiopia. Farmers' cultivars recognized by different names have not been studied as they exist in the production system. These studies on random samples may not enable the capture of co-adapted gene complexes, in the farmers' cultivars, for specific geographical regions and microenvironments. An attempt was made to study farmers' cultivars (Lakew et al., 2000) but only two (Kessele and Mage from north Shewa) were included and the study was also based on morphological or phenotypic characters. A recent study that can be cited for Restriction Fragment Length Polymorphism (RFLP) on barley landrace accessions was that of Demissie et al. (1998) indicating most of the studies on Ethiopian barley germplasm were concentrated on morphological diversity analysis. Lakew et al. (1997) cited few cases where variability studies has been used in breeding programmes in the areas where landraces are adapted and put his view that interest towards the variability of landraces has been academic.

The first successful attempt in the utilization of variation in Ethiopian barley landraces was the systematic evaluation that began in 1988 and resulted in release of line 3336-20 for Shewa and Arsi provinces. Refining of the evaluation procedures is, however, needed to target specific environments, owing to the heterogeneity of barley growing regions. Moreover, the long-term utilization of landraces in a breeding programme

requires crossing with superior pure lines (Ceccarelli & Grando, 1996). Successful crossing requires the estimation of genetic parameters which are very useful in providing information on the inheritance of such characters and help to identify appropriate breeding methods. Such parameters include the subdivision of genetic variances into proportions resulting from additive, dominance and epistatic effects of genes (Falconer & Mackay, 1996). Parameters like heritability, expected genetic advance in response to selection, and the degree of association between characters are also important for the design of a more efficient breeding and selection program (Muehlbauer et al., 1995). Such studies in Ethiopian barley landraces are lacking and the genetic worth of parents for crossing is assessed solely on visual basis.

***Objectives of this study*:**

1) To generate information about the level of diversity within and among the landraces in terms of morphological descriptors, banding patterns of seed storage protein polypeptides, and Amplified Fragment Length Polymorphism (AFLP) so that the farmers' cultivars and accessions can be characterized to facilitate their utilization in the national or regional breeding programme,
2) to assess yield relationships and expected genetic advance that may be achieved through selection within or among landraces,
3) to estimate genetic variances for agronomic characters from crosses involving selected lines from landraces so as to predict the breeding potential and performance of progenies involving these parents, and
4) to assess variability in nutrient accumulation of landraces differing in response to waterlogging, one of the limiting factors for barley production in the central highlands of Ethiopia (north and north west Shewa).

CHAPTER II

REVIEW OF LITERATURE

2.1. Genetic variability, characterization and evaluation of genetic resources

Functionally, genetic resources of crop plants can be divided as advanced varieties in current use and bred varieties no longer in commercial use, primitive folk varieties or landraces, wild or weedy relatives of crop plants and wild species, and genetic stocks such as mutations, cytogenetic stocks and linkage testers (Frankel & Bennett, 1970). According to Frankel and Hawkes (1975), however, the term genetic resources excludes breeding lines and recently released varieties, which are composed of gene combinations rather than the genes themselves.

Genetic resource workers discriminate between "characterisation" and "evaluation". Characterization is an account of the plant's morphology, either throughout its development, or only at maturity and is the recording of information only once on those traits that are highly heritable, easily visible, and expressed in all environments, for example morphological and agronomic characters, and isozyme profiles (Damania, 1990). Characterization provides a standardized record of readily observable morphological characters, together with passport (original) data, to identify an accession in the gene bank. Characterization data are useful in management measures such as the designation of a 'core' and where the primary user is the curator (Frankel, 1989). On the other hand to the plant breeder, this level of characterization is of only limited interest since the characters it covers are no more than subsidiary to those that are his main reasons for using alien germplasm (Frankel, 1989).

Of great interest to plant breeders is characterization which can provide a preliminary account of base line data such as physiological traits responsible for stress tolerance, agronomic traits, cytogenetic information and genetics of host-parasite interactions. Such traits of interest to the breeder are generally less heritable or their determination needs special experimental designs or facilities. The assessment of genetic resources for such traits of economic importance is called evaluation (Frankel, 1989; Damania, 1990). Evaluation is done by growing the materials in different environments, exposing them

to various abiotic and biotic stresses and selecting the best lines for the desirable attributes. Genetic resources merely stored safely are of little value to plant breeders, unless they are evaluated and the resulting data made widely available. Thus, the ultimate goal of any germplasm evaluation is its utilization. The information generated by characterization and evaluation not only improves utilization of the germplasm but also rationalizes storage space by identifying duplicates and eliminating redundant germplasm.

In spite of the wide recognition given to the importance of conserving genetic resources (Frankel & Hawkes, 1975) little is known about the variability in the primitive forms, old landraces, and wild relatives and much work still remains to be done (Damania, 1990). Detailed evaluation of stored populations of different origins allows an understanding of the patterns of variability. The amount of genetic variability among individuals of a variety, population or species is usually thought of as genetic diversity (Brown, 1983). Regional and local patterns of genetic variability are thought to be important to genetic resource conservationists as well as to plant breeders (Jain et al., 1975). Analysis of regional patterns of variability to characters to determine the relative contribution of various regions to genetic resources showed differences in the level of importance of useful germplasm material.

It is commonly believed that genetic vulnerability results from a reduction in genetic variability. Hence, those varieties with a large amount of genetic variability are thought to be less susceptible to the hazards of diseases, insects and other stresses than those with a small amount of genetic variability. This kind of thinking led to the assertion that modern agriculture, using uniform varieties and hybrids, reduces genetic diversity of crop plants and causes them to be more susceptible to biological stresses. Such varieties are also said to be more narrowly adapted than varieties of greater genetic variability. It became evident, however, that genetic diversity *per se* provides no insurance against genetic vulnerability and this has been confirmed in 1916 and 1935 when wheat rust spread throughout the Great Plains destroying hundreds of thousands of acres and numerous varieties of bread wheat, despite the fact that the amount of genetic diversity found among the varieties of wheat in use at the time was considerable (Brown, 1983). This indicates that genetic diversity, in itself, is practically worthless unless it encompasses genes that are useful, either in themselves, or in combination with other

previously evaluated germplasm (Smith & Duvick, 1989). Hence, characterization work should be accompanied by evaluation of materials to identify traits of agronomic importance and traits responsible for stress tolerance (biotic or abiotic), and this should be a priority task of the breeder.

2.2.Measures of genetic diversity in crop genetic resources

Levels of genetic diversity within populations and within species govern rates of adaptive evolution and limit rates of advance in conventional crop improvement. The analysis and characterization of genetic diversity have always been a primary concern of population geneticists and breeders because genetic diversity plays a fundamental role in evolutionary theory and also, without genetic variation there can be no adaptive evolution (Nei, 1990). Both evolutionists, geneticists and agriculturalists are fundamentally concerned with the quality and extent of genetic diversity. There are a variety of measures which may be used to characterize the level of genetic variability in a species and the apportionment of this variation within and between individuals, populations and regions. These measures fall into two classes: i) measures based on genetic variance in quantitative characters which are commonly used in population biology and plant breeding and have the advantage that they are familiar to all scientists in these fields and ii) measures based on allelic diversity at loci level governing qualitative characters. These latter measures have increasing applications due to the development of gel electrophoretic techniques to study allelic frequencies at single loci. The most commonly used measures are:

a) Total number of alleles in the population
b) The proportion of heterozygotes that would be produced if the populations were random mated in which the heterozygosity (H) is given by $H=1-\sum P_i^2$
   where Pi is the frequency on the ith allele at a locus.
c) the Shannon-Weaver information function in which $H' = -\sum P_i \log_2 P_i$.

Measure (a) depends only on number of alleles in the population (allelic richness) while measure (b) and (c) are functions of the frequencies (allelic evenness) as well as the number of the alleles in the populations.

A measure of diversity based on variance analyses of quantitative traits does not allow the direct measurement of the genetic diversity. Such parameters measure only that portion of the genetic variability which is expressed phenotypically. The portion of expressed variability varies markedly with character under consideration and the genetic background and environment in which it is expressed. Consequently, measures of genetic diversity based on variance in quantitative traits may be unreliable indicators of the diversity in a population at the level of the individual gene. Marshal & Brown (1975) considered allelic richness or the number of distinct alleles at a single locus as a basic parameter of genetic variation of a population and more relevant to sampling genetic resources. Even though Marshall & Brown (1975) suggested that measure of genetic variability based on variance analysis of quantitative characters does not allow the direct measure of genetic variability, Shanon-Weaver information index (H') as explained by Jain et al. (1975) has been widely used (Tolbert, 1979; Negassa, 1985, Engels, 1991; Demissie & Bjornstad, 1997) as a measure of phenotypic diversity based on frequency data in barley populations.

Besides the measurement of genetic variability in germplasm resources, genetic relationships as proximity estimation are of importance because breeders can use such information to make decisions concerning the choice of parents to maximize segregation and heterosis (Smith et al., 1990). Estimation of genetic proximity generally involves multivariate statistical methods. A wide array of genetic proximity measures is available and for qualitative locus/allele data, Nei's genetic distance (D) and genetic identity (I) are the commonly used methods. For quantitative morphological characters, the Euclidian distance is frequently used (Manly, 1986). Furthermore, patterns of genetic relationships among germplasm can be summarized using the multivariate methods of cluster analysis and ordination. In cluster analysis, germplasm collections are arranged in a hierarchy referred to as phenogram or dendrogram while in ordination the multi dimensional variability is reduced and depicted in few dimensions. Ordination is more appropriate to elucidate the pattern of variation among germplasm accessions described by quantitative traits (Tsegaye, 1997). Bretting & Widrlechner (1995) recommended principal component and principal coordinates for germplasm management purposes.

2.3. Measures of variability in populations based on morphological descriptors

The role of germplasm in the improvement of cultivated plants has been well recognized (Frankel & Hawkes, 1975). For effective use of germplasm, the breeder would want the required variability to be available in an agronomically desirable background. Germplasm curators as well as breeders have an interest in quantification and classification of genetic variability. Classification based on heterotic grouping was first developed by United States corn breeders (Goodman & Brown, 1988). Many studies in the assessment of genetic variability have focused on genetic markers in the form of gene products such as isozymes (Cox et al., 1988), storage proteins (Cox et al., 1985), and recently DNA markers such as Restriction Fragment Length Polymorphism (RFLP) (Lubers et al., 1991), Random Amplified Polymorphic DNA (RAPD) (Menkir et al., 1997; Bai et al., 1999; Ruas et al., 1999), and Amplified Fragment Length Polymorphism (AFLP) (Ellis et al., 1997; Hayes et al., 1997; Tohme et al., 1996; Breyne et al., 1999). In germplasm collections, such a classification helps designate core collections to enhance efficiency of collection management and utilization. Besides, the description of accessions or germplasm and the recording of information in databases are some aspects of genetic resources work on which request of breeders for material and the ability to manipulate the information in the computer database depends.

Traditionally, genetic distance estimation and classification were based entirely on morphological markers and quantitative traits (Goodman, 1972). Quantitative characters appear less suited as genetic markers because they are often modified by the environment, subject to epistatic and pleiotropic effects, and coded by an unknown number of genes. Besides, because of environmental sensitivity, evaluation data may have little relevance outside the circumstances in which they were obtained. Genotype x environment interaction is a major problem which has yet to be properly addressed (Westcott, 1985) for meaningful interpretation of data in databases. The detailed recording of the test conditions can, however, aid the interpretation and application of the results. Attractive features of quantitative characters may outweigh the disadvantages because: i) databases on germplasm collections or breeders crossing block entries can often be used for clustering (Jein et al., 1975; Spagnoletti & Qualset, 1987), ii) statistical procedures for processing quantitative traits information are readily available, iii) information on quantitative characters adds to an understanding of

ideotype-performance relations, and iv) heterosis may show closer association with distance measures based on such characters or explanation of heterosis may be enhanced by adding morphological measures of distance as another independent variable (Cox & Murphy, 1990).

The International Board for Plant Genetic Resources (IBPGR) has long stressed the importance of passport data as unique identifiers and basic data relating to the origin of accessions. Additionally, the full description of accessions requires their characterization by scoring a limited number of morphological traits which have high heritability, and also the evaluation of more variable traits of interest to the users (Williams, 1989). It is also important to put a high priority on registering all available passport data, including data on altitude, latitude and longitude for landraces so that subsequent associations between passport data and characterization data based on morphological characters can then provide an overall picture of the range of variability in the collections and will also provide a much-needed service to users.

Collections largely consist of landraces with intrinsic genetic diversity within samples and procedures should be designed to take account of this variation and databases designed to accommodate the information obtained. For most crops the language of characterization has been standardized through morphological descriptor lists, but not without much difficulty (Williams, 1989) because there was a need to agree on lists of characters for use in systematic characterization. Therefore, the IBPGR has experienced a sequence of crop-specific descriptor lists developed, then revised and subsequently adapted when used by both curators and breeders. Although the characters considered are more variable in expression, they are generally those of most interest to breeders and include both quantitative and qualitative morphological characters.

2.4. Electrophoretic analysis of storage proteins to measure variability in populations

Within an agricultural community, it is important to be able to recognize and distinguish between different cultivars or varieties of particular crops. This is because cultivars often differ in their quality and other important agronomic characteristics. The methods which are traditionally used to assess cultivar identity and purity vary in detail from crop to crop. The accurate identification of plant material in a gene bank is essential for

effective germplasm characterization. Without such information breeders have no means of selecting appropriate material for entry into breeding programmes. Such identification may be undertaken using traditional morphological characters but this generally involves a lengthy and detailed morphological survey of the field grown plant (Cooke, 1984) and is not always accurate. Moreover, without determining diversity reliably it would not be possible to identify molecular marker/quantitative character associations which have been shown to be useful in the process of germplasm evaluation (Virk et al., 1996). For these reasons a great deal of attention and effort has been paid to the development of laboratory based methods for cultivar characterization using storage proteins as genetic markers.

Seed storage proteins, besides their role in cultivar identification, have been used as genetic markers in areas of analysis of genetic diversity within and among populations, plant domestication in relation to genetic resources conservation, genome relationships especially in polyploid series and as a tool in plant breeding (Gepts, 1990). Protein electrophoretic techniques have become the most widely used in biochemical characterization of plant populations, and have been used in more species, populations and samples than any other technique. Both enzymatic and none enzymatic proteins, generally seed storage proteins, are analyzed for this purpose.

Polymorphism for seed storage proteins have been identified in several species, all or most of which are cultivated species or wild species related to cultivated species. The quantitative parameters of seed storage proteins such as total seed storage protein content is known to be influenced by environment. However, qualitative aspects such as the protein banding patterns after electrophoresis are much less subject to environmental influences. Such biochemical techniques have several potential advantages. Analysis is likely to be much quicker than by traditional techniques and require less personnel (Cooke, 1984) and environmental factors and year of growth have no effect on the electrophoresis of storage proteins (Shewry et al., 1978b; Zilman & Bushuk, 1979; Marchylo & Laberge, 1980;). Studies on maize to quantify the effects of genotypes, source of seeds (the environment in which they were grown), and protein extraction procedure demonstrated that genotypic differences accounted for 60-90 % of the variation, the source of seeds for 10 to 15 %, and the extraction procedure for 2 to 5 % (Smith & Smith, 1986).

2.4.1. Classification of seed proteins

Higgins (1984) defined seed storage proteins as any protein accumulated in significant quantities in the developing seed which on germination is rapidly hydrolyzed to provide a source of reduced nitrogen for the early stage of seedling growth. The system of classification of seed proteins is on the basis of their solubility. According to this classification four categories of proteins occur in seeds: i) albumins, which are soluble in water and comprise mostly enzymic proteins; ii) globulins, which are soluble in dilute salt solutions and generally occur in protein bodies (considered as storage proteins); iii) prolamins, which are soluble in aqueous alcohol solutions and are also found in protein bodies as true storage proteins; iv) glutelins, which are soluble in alkaline or acid solutions, or in detergents and are probably mainly structural proteins although some may have metabolic functions (Lasztity, 1999). In more recent works, classification according to the biological role of the proteins is widely accepted and used. As with the classification of wheat proteins, barley storage proteins (hordein and glutelin) and barley cytoplasmic or metabolically active proteins (albumins and globulins) are also distinguished (Lasztity, 1996).

The proportions of each class of protein present in a seed vary from species to species. Prolamins have only been described in cereals seed and are the major storage protein although oats and rice have high levels of globulin and glutelin type proteins, respectively (Cooke, 1984). In leguminous crops, globulins represent the major part of the seed protein. Although seeds contain metabolic and structural proteins, the major protein fraction consists of the so-called storage proteins which can account for 50 % or more of total protein in the seed (Gepts, 1990). Although a significant difference can be observed between data reported in the literature, it could be stated that the main components of barley proteins are hordeins (25-50 % of the total protein) and glutelins (30-54.5 %). The albumins (3-12.1 %) and globulins (8.4-20 %) occur in lower amounts (Lasztity, 1996). Accordingly, a large part of the work concerning the application of electrophoresis to characterize crop cultivars or germplasm resources has been concerned with the analysis of prolamins (gliadin in wheat, hordein in barley, zein in maize, avenin in oats) or globulins in legumes.

2.4.2. The genetics of seed storage proteins of barley

Seed storage proteins provide an excellent biological model for the study of the molecular and cellular biology of gene expression. The expression of seed storage protein genes is highly regulated both temporally (a specific stage of seed development) and spatially (a specific seed tissue, such as the endosperm in *Poaceae* and cotyledons in *Fabaceae*). Genetic analysis has shown that the electrophoretic patterns of seed storage proteins are generally inherited co-dominantly and in a simple manner, that involves a limited number of genes (Gepts, 1990). The investigation of amino acid sequences of hordein started in the late 1970s and these independently determined sequences were either identical or similar and suggested a high degree of homology with prolamins of other cereals. As a result of progress on the genetics of barley proteins it was revealed that the genes coding for hordein proteins are located on chromosome 5. The polypeptides are coded by separate but linked loci, called Hor-1 and Hor-2. Each locus is a complex multigenic family derived from the duplication and divergence of a single ancestral gene.

Oram et al. (1975) separated hordeins electrophoretically into three groups of bands described as A, B and C hordeins with progressively lower anodic mobilities. It is now generally accepted, however, that hordein polypeptides may be divided into B and C groups. The B hordeins (called also sulfur-rich hordeins due to their relatively high amount of sulfur-containing amino acids) are presented as a mixture of monomers with intramolecular disulfide bonds, but they may form disulfide-stabilized aggregates, too (Lasztity, 1996). B-hordeins are encoded by a single structural locus (Hor-2) located on the short arm of chromosome 5. Two-dimensional electrophoresis (Isoelectric focusing SDS-PAGE) analysis of B-hordein fractions from European barley cultivars with different structural alleles of the Hor-2 locus revealed a total of 47 major polypeptides (Lasztity, 1996), the number of individual polypeptides present in the cultivars varying from 8 to 16. The C-hordeins (sulfur-poor hordeins) account for 10 to 20 % of the total prolamin fraction of barley. SDS-PAGE of C-hordein fractions from different barley cultivars reveals a variable number of major bands with molecular weights between 54 and 60 kDa as opposed to 36 to 45 kDa of B-hordein. Shewry & Miflin (1982, 1983) found a high molecular weight prolamine band, called D hordein, with an Mr by SDS-PAGE of about 105 kDa in European cultivars of barley. A band with faster mobility, or sometimes with two bands, may be present in other lines. D hordein appears to be

encoded by a single structural locus (Hor-3) located about 9 centimorgans (cM) from the centromere on the long arm of chromosome 5 (Shewry et al., 1983).

Genetic analyses have established linkage relationships between several loci controlling seed storage protein electrophoretic patterns or between these loci and loci controlling morphological traits, disease resistance. In barley, the Hor-1, Hor-2 and Hor-3 loci are located on chromosome 5 (Gepts, 1990). Hor-1 and Hor-2 loci, for example, are about 10 cM apart and bracket the *Ml-a* locus, a locus controlling resistance to powdery mildew (*Erysiphe graminis*) (Gepts, 1990). Information on the molecular biology of seed protein genes can be used to improve their nutritional qualities. The nucleotide sequence of genes encoding seed proteins can be modified so that they include a high level of limiting essential amino acids. This requires knowledge of the non-conserved regions in the gene sequence.

2.4.3. Analytical tools to measure seed storage protein diversity of populations

In early studies scientists found that many aqueous solutions, particularly acids, bases, and salts were capable of conducting electricity. The electrical conduction of these substances (electrolytes) was found to be accomplished by chemical changes and the amount of chemical change is proportional to the quantity of electricity (Dunbar, 1987). When one electrolyte is dissolved in water, its molecules dissociate into oppositely charged fragments (ions). Since proteins carry a net charge at any pH other than their isoelectric point, they will migrate and their rate of migration will depend upon the charge density (the ratio of charge to mass) of the proteins concerned. The application of an electric field to a protein mixture in solution will, therefore result in different proteins migrating at different rates towards one of the electrodes and this transport of particles through a solvent by an electric field is referred to as electrophoresis (Dunbar, 1987). Thus, electrophoresis is a technique for separating molecules based on their differential mobility through a solvent in an electric field.

Many analytical methods have been used to characterize protein in general and seed storage proteins in particular. These include centrifugation, affinity chromatography, high performance liquid chromatography (HPLC), amino acid analysis, and various electrophoretic techniques. Electrophoretic separation has contributed more than any other technique to our knowledge of seed storage proteins' variability, partly because of

its rapidity, relatively low cost, and capacity to handle a large number of samples compared to other techniques (Gepts, 1990).

To date, the most widely applied and versatile technique used for laboratory cultivar characterization and diversity studies has been the analysis of seed and leaf proteins and enzymes by various forms of electrophoresis (agarose gel, starch gel, and polyacrylamide gel electrophoresis). The latter two have pores of the same order of size as protein molecules and these do contribute a molecular sieving effect (Dunbar, 1987; Cooke, 1988). However, the success of starch gel electrophoresis is highly dependent on the quality of the starch gel itself, which, being prepared from a biological product, is not reproducibly good and may contain contaminants which can adversely affect the quality of the results obtained. On the other hand, polyacrylamide gel results from the polymerization of acrylamide monomers into long chains and as being a synthetic polymer of acrylamide monomer can always be prepared from highly purified reagents in a reproducible manner provided that the polymerization conditions are standardized. In addition, the polyacrylamide gel has the advantages of being chemically inert, stable over a wide range of pH, temperature, and ionic strength and is transparent (Dunbar, 1987).

Unlike starch gels in which the range of pore sizes obtainable is strictly limited, pore sizes of a wide range can be readily made with polyacrylamide gels. For these and other reasons, polyacrylamide gels have become the medium of choice for electrophoresis although starch gels have been widely used for the analysis of isozymes. There are various types of PAGE and many of these use a version of PAGE originally developed by Bushuk & Zilman (1978).

2.4.3.1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

The vast majority of studies employing zone electrophoresis of proteins in polyacrylamide gels use a buffer system designed to dissociate all proteins into their individual polypeptide subunits (Dunbar, 1987). The most common dissociating agent used is the ionic detergent, sodium dodecyl sulfate (SDS). Polyacrylamide gel electrophoresis in an SDS-containing medium (SDS-PAGE) offers the advantage that all proteins are solubilized, negatively charged, and randomly coiled. The denatured protein is then separated by size or according to molecular weight. By loading with the

anionic detergent SDS, the charge of the proteins is so well masked that anionic micelles with a constant net charge per mass unit result. In addition, the differentiation in the molecular form is compensated by the loss of tertiary and secondary structures because of the disruption of the hydrogen bonds and unfolding of the molecules.

Disulfide bonds which can form between cysteine residues can only be cleaved by a reducing thiol agent such as 2-merchapthoethanol or dithiothreitol. The SH groups are often protected by a subsequent alkylation with iodoacetamide, iodoacetic acid, or vinylpyridine. The unfolded amino acid chain, bound to SDS, form ellipsoids with identical central axes. During electrophoresis in restrictive polyacrylamide gels containing 0.1 % SDS there is a linear relationship between the logarithm of the molecular weight and the relative distance of migration of the SDS-polypeptide micelle (Westermeir, 1997).

There are a number of practical advantages to SDS electrophoresis:

- SDS solubilizes almost all proteins,
- Since SDS-protein complexes are highly charged, they posses a high electrophoretic mobility.
- Since the fractions are uniformly negatively charged, they all migrate in one direction.
- The polypeptides are unfolded and stretched by the treatment with SDS and the separation is carried out in strongly restrictive gels (Westermeir, 1997). Separation by SDS-PAGE has, therefore, become one of the more widely used electrophoretic techniques in seed storage proteins analysis (Gepts, 1990).

Urea has also been used as a dissociating agent which disrupts the hydrogen bonds. The advantage of urea for some applications is that it does not affect the intrinsic charge of proteins and so separation of the constituent polypeptides will be on the basis of both size and charge. The disadvantage is that this combined size and charge fractionation prevents accurate molecular weight determinations. Furthermore, urea is not as good as SDS in dissociating proteins (Dunbar, 1987).

In barley, the biochemical identification of cultivars is concerned with the fractionation of the barley prolamine known as hordein. A substantial amount of knowledge has

accumulated relating to barley seed proteins and enzymes, therefore, there is a large volume of literature concerning the use of protein composition to distinguish between and identify cultivars. Many of the methods used for wheat cultivar identification have been applied to barley. However, modifications are usually required in view of the structural and chemical differences between gliadin and hordein polypeptides. For this reason, early attempts at using a lactate-starch gel electrophoresis system to analyze hordein was only partially successful (Cooke, 1988). However, Shewry et al. (1978) laid the foundation for successful application of a wide range of PAGE methods. From the point of view of routine application, the best methods would appear to be SDS-PAGE with an extraction procedure somewhat simplified from that originally developed by Shewry et al. (1978) and lactate-PAGE in which catalogues of cultivar hordein patterns and taxonomic keys have been produced (Marchylo & Laberge, 1981).

2.4.3.2. Acid polyacrylamide gel electrophoresis (Acid-PAGE)

The International Seed Testing Association (ISTA) recommends that the acid-PAGE method is probably the simplest and most commonly used system for the separation of hordeins from cultivars of barley. It claims that the acid-PAGE system can recognize the existence of different groups of hordein polypeptides, usually termed as A, B, C and D in decreasing order of mobility. Various types of patterns for B and C hordeins in particular have been catalogued. The groups are evident following hordein separation by either acid-PAGE or SDS-PAGE (Cooke, 1988).

The works of Gebre and his coworkers (1986) and Marchylo & Laberge (1981) also demonstrated the potential of acid-PAGE for barley cultivar identification. De Villiers & Laubscher (1989) made evident that the acid-PAGE technique can be used to identify different cultivars on the basis of their hordein electrophoresis patterns and is thus possible to classify a wide range of barley cultivars based on their hordein composition. They also verified the works of Gebre et al. (1986) and Shewry et al. (1978) for the use of a strong reducing agent such as 2 % β-mercaptoethanol (β-ME) or 2 % monothioglycerol to extract hordein proteins completely from barley grain, as many of the bands became more distinct when β-ME was included in the solvent. Doll & Brown (1979) who studied the hordein variation in wild (*Hordeum spontaneum*) and cultivated barley (*Hordeum vulgare* L.) using starch gel electrophoresis was able to resolve the

hordein polypeptides into a banding pattern which was highly reproducible within a given variety but markedly different between varieties.

2.4.3.3. Application of PAGE to assess variability in crop genetic resources

With regard to landraces, evaluation of germplasm collections may take some years and with rapid genetic erosion occurring in many parts of the world, precious germplasm may be lost before additional collections can be made (Damania et al., 1983). Among the various measures of genetic diversity of populations, PAGE of some cereal storage proteins (prolamines) is a valuable tool for gauging variation in populations of landraces and cultivars (Lee & Ronalds, 1967; Doll & Brown, 1979). The application of PAGE of cereal prolamines thus permits a rapid screening of population variation without growing material in the field, and allows identification areas for additional intensive germplasm sampling (Damania et al., 1983). Their work on landraces of barley and wheat from Nepal and Yemen Democratic Republic enabled them to conclude that the variation in storage protein banding patterns revealed by PAGE is considerable and expressed their confidence that the use of PAGE is a valuable tool for assessing variation in germplasm samples.

Because of the fact that barley is a diploid species, unlike wheat which has increased number of prolamine-encoding loci because of the triplication of the chromosomes, the degree of discrimination achieved between barley cultivars is not as great as that observed in wheat regardless of the electrophoresis method used for hordein analysis (Cooke, 1988). The more restricted genetic basis for hordein expression leads to the occurrence of fewer polypeptide bands and a limited number of combinations of these bands (Cooke, 1984). By contrast, in wheat, an increased number of prolamine-encoding loci because of the triplication of the chromosomes leads to the occurrence of more gliadin bands, greater possibilities for mutational divergence and hence more potential differences between genotypes (Cooke, 1988). However, it is generally agreed that the polymorphism between cultivars of barley following SDS-PAGE of hordein is sufficient.

2.5. Isozymes for genetic variability studies in populations

The term 'isozyme' was introduced in 1959 by Markert & Moller to define each one of the possibly many multiple molecular forms of an enzyme. The different molecular forms of an enzyme which are coded by different alleles of the same locus, are called allozymes, now reserving the term isozyme for enzyme forms which catalyze the same reaction but are coded by more than one locus. The genetic basis of isozyme and storage protein variability is one of the reasons why these markers became as widely used as they are for population characterization. Although the characterization and identification can be carried out on the basis of phenotypic differences of electrophoretic band patterns, the advantage of isozymes is that these patterns can usually be interpreted in terms of loci and alleles. Therefore isozymes are ideal genetic markers when estimating genetic variability and characterizing plant populations. The advantages of isozymes over other biochemical markers are:

- allelic expression is generally co-dominant, free of epistatic interactions and usually unchanged by environmental effects;
- alleles of different loci are generally distinguishable;
- enzymatic systems to be studied are usually chosen for technical reasons independent of their level of genetic variability; as a result of this they can represent a random sample of the genome, and
- allelic differences are always detected as mobility differences, independent of the functioning and level of the variability of each enzyme system (Brown & Wier, 1983; Moore & Collins, 1983).

A limiting characteristic of isozyme systems in population studies is the relatively low number of polymorphisms which can actually be observed by gel electrophoresis and specific staining. A comparison of seed proteins and isozyme polymorphisms in *Phaseolus vulgaris*, *Triticum turgidum* var. dicoccoids, and *Hordeum spontaneum* reveals that the high levels of seed storage protein diversity contrast with the relatively low level of isozyme diversity (Gepts, 1990). For example, Doll and Brown (1979) estimated, using sampling theory of neutral alleles, that hordeins are 10 to 30 times more variable than isozymes. Differences in the analytical methods used in studying hordein and isozyme diversity studies, and the fact that seed storage proteins are encoded by multi-gene families that provide additional opportunities for polymorphism

are attributed as reasons why the levels of diversity of isozymes and seed storage proteins are not strictly comparable (Gepts, 1990).

2.6. Molecular (DNA) fingerprinting techniques for diversity study

Over the years the methods for detecting and analyzing genetic diversity have expanded from Mendelian analysis of discrete morphological and cytological variants, to statistical analysis of quantitative variation, to biochemical assays and finally to molecular assays. The science of molecular biology has provided tools to study variation with greater power to resolve different classes of mutational change than previously used methods.

The year 1977 will be marked as the one in which a technique of rapid DNA sequencing, together with the gene cloning technique, revolutionized molecular biology leading to the discovery of many new features of genes such as introns, exons, flanking regions, pseudogenes, and transposons (Nei, 1990). These techniques have also proved to be useful for studying evolution at the most fundamental level of genetic organization, that is at the DNA level. At the DNA level, the extent of genetic variability in populations or among populations can be measured most conveniently by nucleotide diversity which is defined as the average number of nucleotide differences per site between two randomly chosen alleles (Nei, 1987). Within cultivated crop varieties, one can use genetic markers to portray diversity within cultivated germplasm and to identify groupings of cultivars which are adapted to particular regions (Souza & Sorrells, 1989) or perform similarly in crosses to other cultivars (Lee et al., 1989).

Genetic markers have been used to discern evolutionary relationships within and between species, genera, or large taxonomic groupings. Such studies involve studying similarities and differences among taxa using numerous genetic markers. Although phylogenetic trees have previously been established for many species on the basis of isozyme markers and chromosome homology, DNA markers have recently added to the wealth of phylogenetic information available for a number of species (Song et al., 1988; Wendel, 1989; Debner et al., 1990). Using isozymes, substantial genetic maps of some crops had already been assembled by the late 1970s. In principle, visible markers and isozymes are as useful as DNA markers; however, in practice, much greater numbers of DNA markers can be readily found. For instance, crop plants have 10^8-10^{10} nucleotides

of DNA in total and even if a small fraction of these are different between two individuals, an enormous number of potential DNA markers result in contrast to a relatively few visible markers or isozymes which tend to be different between two randomly chosen individuals (Andrew et al., 1991). Therefore, isozyme analysis is not as good as DNA markers due to the lower level of polymorphism and limited number of loci (Bernatzky & Tanksley, 1989)

DNA markers also uncover more differences between individual plants than do protein markers. Proteins are most often detected electrophoretically and allelic variation is dependent on replacement of charged amino acids. However, variation in the DNA coding sequence that results in the replacement of neutral amino acids will not alter the electrophoretic mobility of proteins. It is also possible that some charged amino acid replacements (i.e. a positive charge for a positive charge) will not alter protein mobility appreciably. Nucleotide substitutions at the third base of amino acid codons will not always result in amino acid changes (silent substitutions). Also, variation in non-coding nucleotide sequences such as introns or flanking regions will not alter the final protein product but may result in restriction site changes (Bernatzky & Tanksley, 1989). Therefore, it is believed that the variation in DNA coding sequence can be as much as 10 fold higher than the corresponding protein sequence.

There are two basic types of DNA sequences that are useful as markers. One is derived from mRNA and is known as cDNA. These markers are produced from isolated mRNA that has been enzymatically copied into DNA sequences and cloned into appropriate vectors. These clones therefore represent coding sequences (Bernatzky & Tanksley, 1989). The other type of markers are made from nuclear DNA and are termed genomic clones. Here, total genomic DNA (coding and non-coding) is digested with a restriction enzyme and the fragments are inserted into a vector. These sequences will be both coding and non-coding.

There are advantages to working with both types of clones. The genomic clones are easier to construct than cDNA clones and longer genomic sequences can be selected which make better hybridization probes. However, a large fraction of genomic clones may contain repetitive sequences that produce complex hybridization patterns and these need to be screened out. Many of the non-coding fragments are selectively neutral and

represent sequences that are more rapidly diverging than cDNA clones. Some may lie in regions that are highly polymorphic and can be used to distinguish closely related individuals. Alternatively, cDNA probes have the distinct advantage of representing relatively conserved sequences and this enables them to be used as markers across diverse taxonomic groups (Bernatzky & Tanksley, 1989).

2.6.1. Types of DNA fingerprinting techniques

The fact that large numbers of genetic markers might be found by studying differences in the hereditary DNA molecule itself, revealed as restriction fragment length polymorphisms, was first suggested by Botstein et al. (1980). Recently a range of DNA-based markers have been employed for the study of plant diversity and each method has its own benefits and constraints. Techniques which are particularly promising in assisting selection for desirable characters involves the use of molecular markers such as random amplified polymorphic DNAs (RAPDs), restriction fragment length polymorphism (RFLPs), PCR-based DNA markers such as sequence characterized amplified regions (SCARs), sequence-tagged sites (STS), single polymorphic amplification test (SPLAT), amplified fragment length polymorphisms (AFLPs) and amplicon length polymorphisms (ALPs) using F2 and backcross populations, near-isogenic lines, doubled haploids and recombinant inbred lines (Thottappilly et al., 2000).

Restriction fragment length polymorphism and the PCR-based molecular marker techniques such as RAPD, microsatelites or simple sequence repeats (SSRs) and AFLP are some of the most useful molecular markers for DNA fingerprinting (Thottappilly et al., 2000).

2.6.1.1. Restriction fragment length polymorphism (RFLP)

Among the various markers developed, RFLP was the first to be used in human genomic mapping (Botstein et al., 1980) and they were the first to suggest that large numbers of genetic markers might be found by studying differences in the hereditary DNA molecule itself, revealed as restriction fragment length polymorphisms. Later RFLP was adopted for plant genome mapping (Weber & Helentjaris, 1989).

Restriction enzymes are highly specific "molecular shears" which cleave the DNA at particular sequences (restriction sites). If two individuals differ by as little as a single nucleotide in the restriction site, the restriction enzyme will cut the DNA of one but not the other, generating restriction fragments of different lengths which can then be separated (in an electrical field) and visualized by specific binding of radioactive probe (Andrew et al., 1991).

2.6.1.2. Polymerase chain reaction (PCR) based DNA fingerprinting techniques

The emergence of a new technique (e.g. southern blotting, molecular cloning, pulsed field gel electrophoresis) has transformed the way we think about approaching fundamental and applied biological problems. The capacity to amplify specific segments of DNA, made by PCR represents such a change (Elrich, 1989). PCR is an in vitro method for the enzymatic synthesis of nucleic acid by which a particular segment of DNA with specific sequences can be specifically replicated using two oligonucleotide primers that hybridize to opposite strands and flank the region of interest in the target DNA (Elrich, 1989).

A repetitive series of cycles involving primer annealing, and the extension of the annealed primers by DNA polymerase results in the exponential accumulation of a specific fragment whose termini are defined by 5' ends of the primers. Since the extension products themselves are also complimentary to primers, successive cycles of amplification essentially double the amount of the target DNA synthesized in the previous cycle (Thottappilly et al., 2000). The result is an exponential accumulation of the specific target DNA fragment. RAPD, SSR and AFLP use this method.

Random amplified polymorphic DNA (RAPD)

Williams et al. (1990) was the first to use DNA polymorphism assay based on the amplification of random DNA segments with single primers of arbitrary nucleotide sequences for plants, humans and animals. Since the process uses primers (10bp) the products are easily separated by standard electrophoretic techniques and visualized by ultraviolet (UV) illumination of ethidium bromide stained gel (Williams et al., 1990; Thottapilly et al., 2000). The amplification products will vary in size according to the distance between primer homology with the target DNA. If these distances vary between individuals, then a polymorphism will result. Generally, in this technique, no

prior sequence information is required to design the primers involved in the PCR reaction; hence the term "random" in the naming (Thottapilly et al., 2000).

Microsatelites/Simple sequence repeats (SSR)

DNA sequences with short repeated motifs (2-6bp) are called simple sequence repeats (SSRs) or microsatelites (Hanmada et al., 1982) or second generation markers (Davies, 1993). Microsatelites are based on variable numbers of di-, tri-, or tetra-nucleotide repeats between flanking PCR primers. In other words, SSR polymorphisms reflect polymorphisms based on the number of repeat units in a defined region of the genome being investigated (Weber & May, 1989). In this technique, nucleotide sequence flanking the repeat is used to design primers to amplify the different number of repeat units in different varieties (Thottappilly et al., 2000).

Amplified fragment length polymorphism (AFLP)

AFLP is a PCR-based technology for marker-assisted breeding and genotyping. The technique involves the amplification of small restriction fragments, obtained by cleaving genomic DNA with restriction enzymes, and gel analysis of the amplified fragments (Vos et al., 1995). The rationale of the AFLP technique is based on the use of specifically designed PCR primers that extend into the restriction fragments, amplifying only those fragments in which the primer extensions match the nucleotides flanking the restriction sites. Using this method, sets of restriction fragments may be visualized by PCR without knowledge of nucleotide sequence and the products of the reaction can be visualized by conventional DNA staining or DNA labeling processes using either radioactive or non-radioactive methods (Vos et al., 1995; Thottappilly et al, 2000).

2.7. Genetic variability in Ethiopian barley

2.7.1. History of barley production in Ethiopia

Barley is an important cereal domesticated from wild races found in southwestern Asia. It is a short season, early maturing crop with high yield potential and can be grown where other crops are not adapted. It is a cool season crop and its production is low in the tropics except in the cool highlands as in Mexico, the Andes and East Africa, mainly Ethiopia. Most of Europe, the Mediterranean regions of North Africa, Ethiopia, the Near East, USSR, China, India, Canada and the USA are major production areas of barley.

Barley is a self-pollinating diploid with a 2n=2x=14 chromosomes. Archeological evidence indicated that barley is one of the earliest domesticated crops in the Near East at about 8000 B.C, for example in Syria (Harlan, 1968). The crop was the most abundant grain of the ancient Near East, which was the cheapest, the standard fare of the poor, the ration of the soldier, serf and slave (Harlan, 1976). At present its use, however, has diversified and it is used for different purposes such as food (porridge, bread, roasted grain), for home made beverages, brewing by industries and for animal feeds. In Ethiopia, barley was believed to be cultivated as early as 3000 years B.C (Asfaw, 1989c) and the first Ethiopians known to have cultivated barley are believed to be the Agew people in the north west part of the country (Gamst, 1969).

2.7.2. Morphological variability in Ethiopian barley

The vast range in ecological conditions, the uniqueness and great deal of genetic variability present impressed many germplasm explorers, agronomists and botanists who first observed Ethiopian barley (Qualset, 1975). The crop is one of the oldest cultivated plants and currently, in Ethiopia, landraces comprise the major genetic resources of cultivated barley. The diversity in soils, climate, altitude, and topography together with geographical isolation for longer periods are attributed as the main factors for the presence of large diversity in Ethiopian barley (Harlan, 1968).

A number of studies (Ward, 1962; Tolbert et al., 1979; Bekele, 1983a; 1983b; Negasa, 1985; Demissie & Bjornstad, 1996) have presented the diversity in Ethiopian barley germplasm which were based mainly on discrete (non continuous) characters (Engels, 1991). One of the early studies by Orlov (1929) indicated the uniqueness and marked ecological types of Ethiopian barley significantly differing from the European and Asian origins in which he characterized Ethiopian barleys as having a long tillering period, large number of tillers, long and narrow leaves of milky green color, open flowering, and large grains. Later, studies on the morphological variation (Tolbert et al., 1979) indicated that Ethiopian barley landraces were not more diverse than barley from, for example, Eastern Europe. Cross (1994), however, showed a high degree of variation in agromorphological traits and the Ethiopian landraces were shown to be a very distinct group supporting Orlov's finding. Comparative evaluation between Ethiopian and Iranian landraces (Bjornstad et al., 1997) also revealed that phenotypic diversity was

found to be slightly higher in Ethiopian materials (Shannon Weaver index, H' = 0.52) than Iranian (H' = 0.45) with marked differences in individual characters and this confirmed their distinctness. Engels (1991) put his notion that lower values of diversity reported by Tolbert et al. (1979) could probably be due to the use of growth habit (winter or spring) and awn type (smooth or rough) as these characters do not vary much or at all in Ethiopian barley. The Ethiopian barley landraces are not only distinct in morphological traits but also in terms of resistance genes for several important diseases such as scald (Fukuyama & Tekeda, 1992) and quality traits such as lysine (Lance & Nilan, 1980).

An extensive study by Engels (1991) demonstrated that among the different morphological descriptors, caryopsis type (covered or naked barley) and plant height were the least diverse characters (H'= 0.19 and H'= 0.59, respectively) while spike density caused the highest diversity (H'= 0.86) followed by kernel color (H'= 0.86). It was concluded that the overall diversity index for Ethiopian barley is relatively high for almost all characters as well as for the pooled index over characters (Qualset, 1975; Negassa, 1985; Engels, 1991; Demissie & Bjornstad, 1996) irrespective of the different types and number of characters used by the different workers. A study for individual traits (spike row type, lemma and aleurone color, rachilla hairiness and caryopsis type) indicated that much of the variation was due to variation within accessions rather than regions, altitude classes or agro-ecological zones (Demissie & Bjornstad, 1996). Aleurone color showed significant variation among regions of collection, however.

A clinal association between altitude, row number and rachilla hair type was indicated in the study of barley landraces from Ethiopia (Asfaw, 1989b). The association of six-row and short rachilla in the highlands, two-row and long rachilla in the lowlands was also observed. Demissie & Bjornstad (1996) attributed this association with altitude to linkage with adaptive traits on their respective chromosomes. Despite this association, row type and rachilla are not highly correlated nor chromosomally linked. The altitudinal cline was also ascertained by other findings in Ethiopian barleys. Qualset (1975), for example, found a strong increase in barley yellow dwarf virus (BYDV) resistance with higher altitude. Alemayehu (1995) noted a significant delayed maturity and increased resistance to scald (*Rhyncosporium secalis*) with altitude. This was also

confirmed by Engels (1994) who found a maximum diversity in similar altitudinal range and significant diversity for days to maturity and plant height.

All these findings suggest a basic adaptive process in Ethiopian barley landraces in relation to altitude, encompassing genetic factors on several chromosomes. Demissie & Bjornstad (1996) remarked that genes which are actually adaptive or those which are merely associated through linkage or ancestry, can only be distinguished through analyzing random progenies from appropriate low x high altitude crosses. Moreover, this analysis highlights how useful good evaluation data may be for analyzing characterization data. For ease of understanding, frequencies of characters observed in Ethiopian barley compared to world-wide distribution is presented in Table 2.1 below as summarized by Qualset (1975).

Table 2.1. Frequency of characters observed in Ethiopian barley compared to world-wide distribution (Qualset, 1975).

Character	Description	Number of collections	Frequency (%)	
			Ethiopia	World
Spike type	6-rowed	430	66.0	71.0
	2-rowed	33	5.1	25.1
Grain color	White	268	41.2	55.1
	Blue	177	27.2	36.7
Rachilla hair	Long	175	26.9	60.9
	Short	476	73.1	39.1
Glume awn	Long	374	57.6	55.2
	Short	275	42.4	44.8
Spike density	Lax	601	92.6	–
	Dense	48	7.4	–
Caryopsis	Covered	572	87.9	87.5
	Naked	79	12.1	12.5
Heading time	Early	400	63.4	–
	Midseason	149	23.6	
BYDV reaction	Susceptible	511	78.5	98.0
	Resistant	140	21.5	2.0

Because of such tremendous morphological diversity, Vavilov (1926) suggested that Ethiopia was a center of origin of barley. However, due to the absence of a wild progenitor *Hordeum spontaneum*, others consider Ethiopia as a secondary gene center or secondary center of diversity and not as center of origin (Tolbert et al., 1979), an

accumulation center (Schieman, 1951) or center of concentration (Ward, 1962). Bekele (1983b) and Negassa (1985) claimed, however, that Ethiopia might be a center of origin of barley as was originally suggested by Vavilov (1926).

2.7.3. Molecular and biochemical variability in Ethiopian barley

Earlier population studies have revealed that Ethiopian barley landraces are diverse both in terms of 5 isozyme (Bekele, 1983a; 1983b) and storage protein loci (Asfaw, 1989a; 1989b). Unbiased genetic identity and genetic distance estimates between 51 Ethiopian barley populations confirmed the high degree of differentiation among the populations (Demissie & Bjornstad., 1997). Recent study of the diversity of barley population using molecular markers is that of Demissie et al. (1998). In his study he included 43 landraces in set 1 represented by single line and the second set consisted of 65 lines of different geographical and altitudinal origins. His work revealed that at the probe level, certain fragments confined to certain geographical regions could be distinguished. However, more often no regional trend was apparent, as when the same uncommon band occurred in a few landraces from widely different regions. Also the genetic distance estimates between regions are rather low. Based on their DNA polymorphisms, barleys from Arsi and Bale regions may be considered as one source of germplasm and Shewa appears distinct. A further comparison of genetic similarity and genetic distance estimates showed that in spite of an altitude span from 1650 to 3750 masl, the estimated differences between altitude classes were slight, indicating an apparent lack of genetic differentiation linked to altitude. Similarly comparison of RFLP bands among agroecological zones revealed that genetic differentiation was very small, reflecting the absence of variations between the zones (Demissie et al., 1998).

2.8. Causes of variation in Ethiopian barley

Genetic variation within plant species is a product of three kinds of interacting factors: biotic, abiotic and species characteristics like population size, mating system, mutation, migration and dispersal. Through complex interplay and jointly with selection and random effects, these factors affect the genetic composition of populations (Frankel et al., 1995). Harlan (1975) indicated that variation in centers of diversity could be attributed to human, environmental factors, and the dynamic processes of hybridization, segregation and selection. Interactions involving the biotic factors (host-pathogen interaction) and physical environmental variables, all play a significant role in evolution

and adaptation. Both of the physical and biotic environmental factors exert selection pressure on variants leading to the emergence and adaptation of specific types. Therefore, in heterogeneous environments the adaptation of genotypes differ depending on locations.

The highly heterogeneous Ethiopian environment, particularly of the "barley belt", owing to its low latitude and high altitude has been pointed out as a key factor for diversification. Such environment which created favorable grounds for natural selection to act upon must have been augmented by the breeding system of *H. vulgare* L (predominantly self-pollinating, with some out-crossing) which facilitated both fixation of types and occasional genetic recombination (Briggs, 1978). Social factors (social values as criteria for selection, diversified uses and association between barley types and uses) are also part of the causes for the diversification. Thus the presence of the morphological, biochemical and molecular groups in Ethiopian barley are the results of accumulated long-term mutations, hybridization, gene recombination and natural and human selection operating in heterogeneous environments.

2.9. Utilization of the diversity of Ethiopian barley germplasm

2.9.1. International use

The presence of domestic varieties of barley in Ethiopia was acclaimed by many 19th century crop taxonomists. Though largely fragmented, early studies on Ethiopian barley covered many aspects including morphology, agronomy, ecology, diversity, evolution, genetics and taxonomy (Asfaw, 1996). Interest then shifted towards utilization of Ethiopian barley germplasm in the breeding and development of modern barley varieties. Harlan (1968, 1969) considered Ethiopian barley to be inferior by modern standards of cultivars. This claim was based on observations made when the material was grown far away from its natural habitat and geographical range. Despite his claim, very valuable sources of genetic material have been identified for breeding and have significantly contributed to the understanding and improvement of the crop world-wide.

Besides, it has been documented that the release of a few successful new cultivars over large areas has led to the displacement of many old cultivars, many of which may possess useful genes for future need (Cecarelli & Grando, 1996). This is typically a feature of plant breeding in developed countries for favorable environments in which

the narrowing of the genetic base is accompanied by a trend toward homogeneity (Simonds, 1983).

Landraces are typically mixtures of different genotypes and in self pollinated crops they are mixtures of probably a high number of homozygous genotypes which contain a large amount of genetic variation within adapted genetic background in which farmers are basically interested. In the case of barley, a self-pollinated crop, this genetic variation is readily useable. Landraces are, therefore, potentially useful in current and future plant breeding programs.

Several characteristics of Ethiopian barley landraces (tillering, straw strength, kernel size, disease resistance and high protein content) were believed to be important from a plant breeding point of view (Qualset, 1975). Other useful characteristics of Ethiopian barley landraces include tolerance to marginal soil conditions, tolerance to insects (barley shoot fly and the Russian wheat aphid) and frost, vigorous seedling establishment and quick grain filling (Gebre & Alemayehu, 1991).

High resistance to powdery mildew, loose smut, leaf rust, net blotch, septoria blotch, scald, barley yellow dwarf virus (BYDV), and stripe mosaic virus has been found in Ethiopian barley landraces (Harlan, 1976). Resistance to BYDV that caused severe yield losses on barley in California was found only from Ethiopian materials (Qualset, 1975). The genetic analysis showed that resistance to BYDV is conferred by one gene on chromosome 3 (Rasmusson & Schaller, 1959; Schaller et al., 1964) and the resistance was found to be easily transferred by back crossing. Schaller et al. (1963) found that about 21 % of barley from Ethiopia were resistant to BYDV and an overall frequency of 17.5 % of major collections by other explorers (Qualset, 1975).

Qualset and Suneson (1966) constructed a breeding population which was developed to incorporate many of these characteristics of Ethiopian barley germplasm into adapted genotypes. Besides the presence of protein as high as 18 % and lysine level of 4.4 % of protein in Ethiopian barley landraces drew considerable interest of researchers internationally (Qualset, 1975; Harlan, 1976).

2.9.2. Utilization by the national breeding programme

Despite the significant contribution of Ethiopian barley landraces internationally, impressive progress has not been made in the use of the various locally adapted landraces by the national breeding programme. This was partly because of the fact that early breeding programs focused mainly on introductions and evaluations of exotic materials under optimum management conditions to develop food and malting barleys (Gebre & Alemayehu, 1991). Accordingly, adoption of food barley releases by peasants was rather poor because under traditional management practices, the farmers' landraces performed equal or better than the new cultivars. Hence barley is grown mostly as landraces in all regions by subsistence farmers with little or no application of fertilizers, pesticides, and herbicides.

Another barrier to the utilization of landraces in many developing countries was the misconception that they have low yield potential and are susceptible to diseases. Many plant breeders were also reluctant to devote a greater part of their resources for the exploitation of landraces and wild species in the past. This was because the potential value of this germplasm for stressed environments was not fully appreciated. Besides, the breeding objectives of the developed countries are different from those of the developing countries. Advanced breeding programs in developed countries began utilizing exotic landraces long ago and have fully exploited them so they no longer seek variability but only single genes from the wild relatives and grasses of the tertiary gene pool (Damania, 1990). The breeding objectives of developing countries, on the other hand, are to develop varieties adapted to withstand harsh environments and low inputs. So the use of selections from landraces and crosses with wild and primitive forms to produce well-adapted germplasm for targeted agroecological zones is imperative.

Recent evaluation of cereals by many scientists working in different countries who were searching for economically useful genes or gene combinations expressed confidence that such materials are a usable source of breeding stocks, although they still require thorough assessment (Damania, 1990). The presence of individual genotypes within Ethiopian barley landraces which have better yield and more desirable expression of agronomic characters than the original landraces has been confirmed recently (Lakew et al., 1997).

2.10. Genetic erosion and role of farmers in conserving genetic diversity

2.10.1. Risk of genetic erosion in barley

While crop evolution is a continuous process of the appearance and disappearance of specific forms, the diffusion of improved varieties into areas of traditional agriculture and genetic diversity is thought to introduce a new and more determinant element into evolutionary system. The result is genetic erosion, an accelerated loss of germplasm from the extant crop gene pool, so that more germplasm is lost than is replaced by natural processes or by the introduction of new germplasm. The assumption is that a combination of modern conditions, including new technology, rapid population growth, and rapid economic and cultural change create a fundamentally new environment (Stephen, 1991). This new environment is assumed to be antithetical to maintaining elements from the previous evolutionary systems such as the ancient crop germplasm of landraces.

An example is the hooded barley that was reported to be characteristic in Ethiopia and recovered from Ethiopian barley germplasm samples kept in foreign gene banks. As selection pressures favored the preservation of many botanical forms, it also disfavored this hooded type of barley which became less and less frequent and even extinct from cultivation (Asfaw, 1996). Some degree of frustration was also reflected from development agents and farmers themselves that certain barley types common in the fields of farmers at one time are becoming very rare. Replacement of barley by crops such as bread wheat, teff and oats has been attributed as another cause of genetic erosion of barley in the Ethiopian conditions (Engels & Hawkes, 1991).

A large proportion of the worlds' population depends on genetic resources produced in a few regions and by a relatively small number of farmers. Diffusion of crops between continents, modern breeding techniques, and increasing reliance on modern crop varieties contribute to the importance of crop genetic resources that are found in specific regions of biological diversity (Stephen, 1991). The need to conserve crop germplasm has been recognized since the work of Vavilov (1926) and urgency of conservation has increased as greater numbers of people rely on modern varieties of a few crops. Since plant breeding is essentially a process of exploitation of genetic variability, breeders could also examine means of conserving the already existing genetically variable germplasm as well as creating new varieties.

2.10.2. Role of farmers in conserving genetic diversity

It goes back to 10,000 years that man, especially woman, discovered that seeds could be grown near to home to reduce the effort required in gathering food and eventually they were changed from hunter gatherers to farmers. Early farmers initiated a series of partly conscious selections that have resulted in the landraces that are seen today. Because these landraces have been selected in the local habitat for thousands of generations, they are consequently adapted to their specific environment (ICARDA, 1996).

In Ethiopia as well as in many other developing countries, farmers play a central role in the conservation of germplasm, as they hold the bulk of genetic resources. The small-scale farmers always retain some seed stock for security unless circumstances detect otherwise. Germplasm conservation by farmers to maintain genetic variability of coffee in Ethiopia has been reported (Werede, 1991). Farmers often plant populations of local types on small areas usually for safety purposes alongside the more uniform coffee berry disease (CBD) resistant lines distributed as improved varieties. Maintaining germplasm in this way will provide an additional support to *ex situ* measures of landrace preservation long-term protection against extinction of native cultivars.

Over many seasons, and probably human generations, farmers were accustomed to select seeds to be saved for the next crop, and eventually realized the value of saving from the most productive plants. They did not simply preserve seeds from the best plants each year, but maintained a mixture of types. Such risk avoiding activity was essential for farmers who needed to feed their families on what they grew.

2.11. Genetic variance, heritability and combining ability of quantitative characters

2.11.1. Genetic variances

Genetic variation in quantitative characters in plant populations are of prime concern to a breeder and their quantitative characterization is emphasized because such information is important in designing effective breeding programs. Genetic improvement of quantitative characters is based on effective selection among individuals that differ in genotypic values. The variation among genotypic values represents the genotypic variance of a population. A description of the various types of gene action that determine the genotypic value of individuals in a population will be

helpful in understanding the concept of genetic variance (Fehr, 1987). Unlike qualitative characters, the difficulty with quantitative characters is that they tend to be continuous in their variation and application of classical Mendelian analysis is difficult or impossible because the genotypic classes cannot be distinguished.

In earlier years, phenotypic variability was the only criterion to judge the available variability. This was inadequate, since environmental and genetic variabilities, in the absence of progeny tests, were confounded. Interest in quantitative genetics developed in the 1940s following the work of Comstock & Robinson (1948) and Mather (1949). Developments in this area allowed for the partition of either means or variance which provide information as to the presence or absence of genetic variability and, in addition, provide information on the type of gene action involved. The primary objective of measuring phenotypic variation is to partition it into components attributable to the genetic and environmental causes of variation. It is the relative magnitude of these components which determines the genetic properties of populations, particularly the extent to which various relatives resemble each other. Partitioning of the variance into its components allows estimation of the relative importance of the various determinants of the phenotype (Falconer & Mackay, 1996). The total variance is the phenotypic variance or the variance of the phenotypic value. Partitioning of the phenotypic variance into genotypic and environmental variances does not reveal the causes of resemblance between relatives. Hence, the genotypic variance must be further separated into three components: that due to additive effects of genes, dominance deviations from the additive scheme, and that due to deviations from the additive scheme attributable to inter-allelic interactions (Warner, 1951). To generalize, the genetic parameters of interest are the additive and non-additive (i.e. dominance and epistasis) genetic components of the genotypic variance. There are various models or mating designs developed that can be used by geneticists and plant breeders for the estimation of genetic variances. Three of the more commonly used mating designs are the diallel, design I (nested design), and design II (factorial design) and a primary criteria of all these designs is that individuals evaluated from a population be a random sample of all possible genotypes. Any model developed for the estimation of genetic variances involves biological assumptions which vary somewhat with model but the more common restrictions are 1) normal diploid behavior at meiosis, 2) no maternal or cytoplasmic effects, 3) no multiple alleles, 4) linkage equilibrium, and 5) no epistasis

(Sprague, 1966). The mating designs differ in the genetic material evaluated, which determines the extent to which additive, dominance, and epistatic variances can be estimated (Fehr, 1987). However, the estimation of the additive and non-additive genetic components can be made from the experimental material in terms of general and specific combining ability variances (Griffing, 1956a).

2.11.2. Heritability of traits

Burton & De Vane (1953) defined heritability as a measure of the efficiency of the selection system in separating genotypes. The concept of heritability originated as an attempt to describe whether differences actually observed between individuals arose from the differences in the genetic makeup between individuals or resulted from different environmental forces. Discrepancy among research workers was observed as to how heritability should be defined when used in relation to self-fertilizing crops. It is assumed reasonable to think of heritability as a measure of the degree to which the phenotype reflects the genotype. Heritability could be estimated 1) in broad sense where the numerator of the ratio contains the total of the genetic variance which arises due to genetic effects; 2) in the narrow sense where the numerator contains only the additive genetic variance; or 3) as a ratio whose numerator contains less than the total genetic variance, yet more than the additive genetic variance (Rasmusson & Glass, 1967).

Genes segregate and come together in new combinations exhibiting intra-allelic interactions (dominance) and inter-allelic interactions (epistasis). The differences between the actual effects of genes in combination and their average effects in the population are dominance and epistasis effects which are transmitted only in part. Thus heritability in the broad sense considers total genetic variability in relation to phenotypic variability, while heritability in the narrow sense considers only the additive portion of the genetic variability in relation to the phenotypic variability (Hanson, 1963). The additive portion of genetic variance reflects the degree to which the progeny are likely to resemble the parents and heritability in this sense denotes the additive genetic variance as percent of the total variance (Robinson et al., 1949). The techniques for estimating the degree of heritability in crop plants fall into three main categories namely parent-offspring regression, variance components from analysis of variance and approximation of non heritable variance from genetically uniform populations to estimate total genetic variance (Warner, 1951).

In barley quite a number of studies have been done to estimate heritability of characters and to investigate if increases in grain yield could be obtained by selecting among segregating progenies for the morphological components of grain yield. Selection for yield through components was very effective in certain situations, but could not be recommended as a routine procedure before a careful study of the relationships between yield and the components in the parental varieties was assessed (Rasmusson & Cannell, 1970).

Vazequez and Sanchez-Monge (1989) in the analysis of F1 and F2 diallel crosses showed that additive and dominance effects were highly significant for internode length. Boukerrou and Rasmusson (1990) reported heritability values of 0.75, 0.76, and 0.67 for biomass, vegetative biomass and grain yield, respectively. A study involving 2-rowed and 6-rowed barley types indicated that heritability was high (0.82-0.83) for 1000-grain weight in 2-rowed types and 0.88-0.97 for grain number per ear in 6-rowed ones (Vaculova, 1991). In a comparative study of 6-rowed and 2-rowed barley types, high heritability estimates were found for ear length, plant height, and grain yield in 6-rowed types and for plant height and grain yield in 2-rowed types (Nadziak et al., 1994). In a study comprising segregating populations, higher heritability estimates were observed for days to maturity than for days to heading (Singh & Singh, 1990a), and very low heritability (0.26-0.37) for harvest index (Theoulakis et al., 1992). High broad sense heritability and genotypic coefficient of variation for grain yield (Marocco et al., 1992), and highest genotypic coefficient of variation for grain yield per spike and grain yield per plant but lowest values for plant height and 100-kernel weight were reported (El-Hennawy, 1997). The latter work also illustrated heritability values ranging from 29 % for harvest index to 71 % for grains per spike. Because of high genotypic coefficient of variation and high heritability values for grains per spike, the expected genetic advance for this character was also the highest (58.13 %) and lowest (11.62 %) for plant height (El-Hennawy, 1997). El-Seidy (1997) on the other hand obtained high values of broad sense heritability for heading date and kernels per spike, moderate values for spike length and 1000-kernel weight, low to moderate values for spikes per plant and low values for grain yield per plant. Broad sense heritabilties in the range of 42 to 86 % were also reported for heading date (Martinez & Foster, 1998).

Non additive gene action was found to play the predominant role in the inheritance of grain yield per plant and plant height whereas additive genes were more important in the inheritance of grain number per ear, ear number per plant and 1000-grain weight (Kudla & Kudla, 1995).

2.11.3. Combining ability

The concept of combining ability is very important in plant breeding especially in combination with testing procedures in which it is desired to study and compare the performances of lines in hybrid combinations (Griffing, 1956b). The choice of parents for inclusion in a crossing program is of particular importance to any breeding program. Since the development of parents for production of hybrids by the male sterile method requires considerable time and effort, it is desirable to have estimates of combining ability early in the breeding program (Gyawali et al., 1968). Diallel crossing has proved to be of considerable value to plant breeders in making decisions concerning the selection of breeding materials that show the greatest promise for success (Gardner, 1966).

Gilbert (1958) has listed the assumptions needed for diallel analysis, following Hayman's (1954) method, as diploid segregation, no reciprocal differences, independent action of non-allelic genes, no multiple allelism, and genes independently distributed at random between the parents. Sprague and Tatum (1942) employed the terms general combining ability to designate the average performance of a line in hybrid combination and specific combining ability to indicate those causes in which certain combinations do relatively better or worse than would be expected on the basis of the average performance of the lines involved. The diallel analysis of Griffing (1956a) following Yates (1947), being made in terms of combining ability, has less demanding genetic assumptions and is probably preferred (Mayo, 1987).

2.12. Association of quantitative characters in barley

2.12.1. Correlations among quantitative characters

Correlation in plant traits may arise from the genetic effects (which could be pleiotropy or linkage) and environmental factors (Falconer, 1990). Simultaneous variation in two or more characters arises as a result of genes that have pleiotropic effects upon segregation (Finne et al., 2000). The genetic correlation arising from pleiotropy expresses therefore the extent to which two characters are influenced by the same genes. Environmental correlations reflect a similarity or dissimilarity in the response of traits to a common environment (Falconer, 1989). Three types of correlations in plant traits are distinguished: 1) phenotypic correlations which measures the extent to which the two traits observed are linearly related; 2) genotypic correlation which measures the extent to which the same genes or closely linked genes cause covariation in two different traits and 3) environmental correlations which arise because the same environment is causing simultaneous variation in both traits (Singh, 1991). Cheverud (1988) studied the relationship between genetic and phenotypic correlations and found that much of the dissimilarity between phenotypic and genetic correlation estimates seems to be due to imprecise estimates of genetic correlations. Hence, he concluded that when reliable genetic estimates (correlations) are unavailable, phenotypic correlations may be substituted for their genetic counterparts. Willis and Coyne (1991), however, re-examined his data and found no support for Cheverud's (1988) assertion that phenotypic correlations reflect genetic correlations.

Knowledge about the sign and magnitude of genetic correlations are important both for understanding the relationship between quantitative characters and fitness in natural populations and for predictions of correlated responses to selection in breeding programs (Falconer, 1989). If genotype by environment interaction exists, greater differences are expected to occur in the estimates of genetic correlation coefficients obtained in different environments. Hence, based on the variance and covariance components for genotype x environment interaction a new type of correlation coefficient called genotype x environment correlation is suggested (AAstiveit & AAstiveit, 1993). Bouzerzour & Dekhili (1995) noted that genotype x environment interactions, particularly related to seasonal effects, seriously limits selection for increased barley grain yield and the effect was to reduce the genetic variance component, heritability estimates and genetic correlation coefficients. It is also

suggested that selection for yield and yield components in high yielding locations does not identify genotypes suitable for low yielding environments (Ceccarelli et al., 1992; Bouzerzour & Dekhili, 1995). The existence of traits that are desirable under drought and undesirable under favorable conditions was also reported (Shakhatrel et al., 2001).

Considerable correlation studies between agronomic characters and grain yield in barley have been done to determine the principal components influencing final grain yield depending on the environment in which the materials were evaluated. Hadjichristodoulou (1990) in a dry environment found that among the traits of barley, 1000-grain weight was the most stable and grain yield the most variable trait. In 6-rowed barley, 1000-grain weight was positively correlated with grain yield, straw yield, total biological yield and plant height (Hadjichristodoulou, 1990). Negative and non significant correlations of 1000-grain weight with plant height and shoot dry weight at low level of N nutrition, but positive and non significant correlations at the higher N level was reported (Singh et al., 1993). Shoot dry weight had positive and significant correlations with plant height, number of tillers and leaves per plant, and grain yield at both levels of N. Its correlation with 1000-grain weight was negative and non significant at low levels of N, but non significant and positive at the higher level of N.

Correlation coefficients between grain yield and harvest index and between grain yield and biomass in F3 and F4 generations were observed to be high and very high, respectively (Theoulakis et al., 1992). Selection for harvest index in the F3 generation showed positive effects on harvest index in the F4 generation but its contribution to grain yield was not significant. In F1 hybrids and their elite parent lines, grain yield per plant was significantly and positively correlated with spikelets per ear, tillers per plant and 1000-grain weight. The association between tillers per plant, spikelets per ear and 1000-grain weight were also positive and significant (Singh et al., 1998). Significant and positive correlation coefficients amongst grain yield, biological yield, straw yield, plant height and harvest index were expressed in dry and wet testing environments. Correlations among days to heading, days to maturity and grain yield were significant and positive only at the driest location, however, which suggested that different phenologies are required to maximize grain yield in wet and dry environments (Shakhatrel et al., 2001).

2.12.2. Path coefficient analysis

Although statistical correlations between agronomic characters and grain yield are helpful in determining the principal components influencing final grain yield, they provide an incomplete representation of the relative importance of the direct and indirect influences on the individual factors involved (Bhatt, 1973). This is especially true for cereals because yield components in these crops occur successively and may therefore interact in compensatory patterns during plant development (Garcia et al., 1991). In wheat, for example, number of spikelets is determined at time of floral initiation, but variation in number of florets per spikelet can occur later. In barley, spikelet production can extend for a longer period, but there is only one floret per spikelet. In both species, however, the upper limit of kernel number is determined before head emergence (Rasmusson & Cannell, 1970). A consequence of differing times of determination of components and their variable responses to environmental changes is the well known compensatory effect which yield components show. Adams (1967) has cited several examples of these effects and pointed out that such component compensation leads to negative correlations between components. The correlation data from barley experiments conducted by Rasmusson and Cannell (1970) provided additional evidence of the important effect environmental variation has on the relationships among yield components.

Path coefficient analysis as used and elaborated by Dewy & Lu (1959) is a standardized partial regression coefficient which measures the direct influence of one variable upon another and allows the separation of the correlation coefficient into components of direct and indirect effects. Thus, a path coefficient analysis provides a measure of the relative importance of each independent variable to the prediction of changes in the dependent one. The use of the method requires a cause and effect situation among the variables and based upon experimental evidence direction in the causal system must be assigned (Dewy & Lu, 1959).

Path coefficient analysis has been used successfully to clarify the interrelationships between yield and many characters in crested wheat grass (Dewy & Lu, 1959); wheat, *Triticum aestivum* L. (Bhatt, 1973; Gebeyehu et al., 1982; Belay et al., 1993). This method of analysis has also been used in barley to study the relationships between grain

yield and yield components (Singh & Singh, 1990b; Garcia et al., 1991; Mandel & Dana, 1993; El-Hennawy, 1997; Naik et al., 1998; Verma et al., 1998).

It is generally accepted that a selection program based upon the components of yield may fail to accomplish the goal of improving yield because the components of yield are greatly influenced by various environmental factors and stresses (Adams, 1967; Rasmusson & Cannell, 1970). Therefore, it is important that the plant breeder shall study and test various models involving yield components in the environmental niche where a breeding program is to be established. Accordingly the parental cultivars for crossing shall be selected based on the components of yield. In Ethiopia, as far as literature shows, such research is lacking except the work by Alemu (2001) and Sinebo (2002) on landraces and introductions.

2.13. Landraces and environments: a case of waterlogging stress and soil burning (guie) barley production system in north Shewa, Ethiopia

2.13.1. Characteristics and extent of waterlogged soils under "guie" in Ethiopia

The major *guie* soils (soils productive only after burning) of Ethiopia are found in Shewa (central Ethiopia), although few also occur in Wello, Arsi and Kefa administrative zones. The approximate extent of these areas is 540 000 ha. Such soils mainly on Shewa highlands occur approximately between 2000 and 3000 masl. The predominant physiography is a highland plain plateau. These areas receive between 1100 and 1500 mm of annual rainfall, of which 70-80 % comes during the heavy rainy season, from June to September. Consequently, such soils tend to get waterlogged, thus resulting in low permeability and poor internal drainage. This leads to poor aeration, restricted root growth and hence low productivity (IAR, 1979).

2.13.2. Soil aeration and root and shoot growth under waterlogged soils

Soil aeration

Crop plants require a free exchange of atmospheric gases for photosynthesis and respiration. Plants can be easily suffocated if this gas exchange is impaired. This most common impediment to gas diffusion is water that saturates the root environment in poorly drained soils or that accumulates above soil capacity as a result of excessive rainfall. In waterlogged soils, air is displaced from pore spaces either to different depths of sub-soil (which results in high water table) or in the top soil.

Since oxygen and other gases diffuse in air about 10^3-10^4 times more rapidly than in water or water-saturated soils (Armstrong, 1979), oxygen is depleted more or less rapidly by the respiration of soil microorganisms and plant roots in waterlogged soils. Various degrees of oxygen depletion (hypoxia) and anoxia (the absence of molecular oxygen) occur. Once molecular oxygen has been consumed in respiration, various populations of microorganisms utilize other terminal electron acceptors for respiration (Marschner, 1986). The change from oxygen sufficiency to deficiency can occur within a few millimeters, and even in aerobic soils the interior of soil aggregates may be anaerobic. The reduction of oxygen below optimal levels, termed hypoxia, is the most common form of stress in wet soils and occurs during short-term waterlogging (Figure 2.1) when the roots are submerged under water but the shoot remains in the atmosphere.

Figure 2.1. Barley field at Sheno Research Center demonstrating waterlogging due to continuous rainfall in the main season.

As free oxygen is depleted nitrate is used by soil microorganisms as an alternative electron acceptor in respiration. Nitrate is reduced to nitrite (NO^-_2), various nitrous oxides (eg., N_2O, NO) and molecular nitrogen (N_2) in the process of denitrification (Marschner, 1986). Nitrite can accumulate temporarily during this process, especially in soils that are alternatively wet and dry.

Root and shoot growth

Short-term responses of plants to anaerobic soil conditions can readily be demonstrated by waterlogging of previously well-aerated soil. Since the direct effect of waterlogging is a major decrease in the gas exchange between the atmosphere and the soil, root growth and function are likely to be inhibited long before O_2 is exhausted from the soil water (Drew, 1990). In soils temporarily water-saturated or in fields with a high water table, roots grow only in a small region near the surface and do not exploit as large a soil volume as they would under aerated conditions. This makes them more susceptible to subsequent droughts and increases their fertilizer requirements (Armstrong, 1978 cited by Marschner, 1986). Since growth processes do not continue without oxygen, growth of existing roots ceases immediately and they may die within a few days. In contrast, shoot growth in terms of dry weight increase continues for several days at a similar or an even somewhat higher rate, although visible symptoms of waterlogging injury (transient wilting, inhibition of leaf extension, and chlorosis) are observed within a few days (Trought & Drew, 1980a).

Most plant species not adapted to waterlogging (non wetland, mesophytic species) develop injury symptoms sequentially over a period of several days if waterlogging continues. Wilting, leaf senescence and, in herbaceous species, epinasty (downward bending of leaves) are likely to be the first symptoms. A decrease in the hydraulic conductivity of the roots and an accumulation of ethylene in the shoots are responsible, respectively, for wilting and epinasty. A rapid decline in or cessation of shoot extension growth is another typical symptom, followed after several days of waterlogging by enhanced senescence of the lower leaves, indicating nitrogen deficiency or lack of root-borne cytokinins (Drew, 1990).

The severity of the effect of waterlogging on growth and yield depends on the plant species, developmental stage of the plants, soil properties (eg. pH, organic matter

content), and soil temperature in particular. For instance, waterlogging of wheat for 30 days during grain filling at soil temperature of 15 and 25°C reduced grain yield by about 20 % and 70 %, respectively (Luxmoore et al., 1973). In barley, it has been observed that saturated soil conditions will adversely influence growth within one week. Growth reduction will depend on the duration of flooding and the stage of development of the plant at the time of waterlogging. Yu et al. (1969) reported reduced leaf formation, reduced stem elongation and delayed heading following 17 days of waterlogging, but no difference in final yield. Bourget et al. (1966) found that the two-leaf stage of barley was most susceptible to waterlogging injury and that most of the damage to yield capacity occurred in the first seven days of waterlogging. Despite the greatest reduction in dry matter of barley when waterlogged at an early developmental stage, young plants at initiation of waterlogging tend to recover their growth rates following periods of waterlogging whereas no such recovery occurred in barley waterlogged at stem elongation stage of development (Bourget et al., 1966). Assefa (1997) also demonstrated that waterlogging of the spring barley cultivar Blenhgim at three leaf stage for eight or 12 days had on average 65.8 % and 57 %, respectively, of the dry weight of the control plants and this effect of waterlogging on dry matter accumulation continued throughout the growth period of the plants and dry matter determined at grain filling period were still significantly less than the control plants.

Whether the principal cause of waterlogging injury to the shoots is related directly to oxygen deficiency in the roots or more indirectly to the production of toxic substances in the soil depends on circumstances. In soils high in organic matter and nitrate, sudden waterlogging might lead to an accumulation of nitrite in the soil solution to concentrations that are toxic to the roots of sensitive plant species (Marschner, 1986). Waterlogging injury caused primarily by manganese toxicity occurs in plant species inherently low in tolerance to manganese, especially in acid soils containing high levels of manganese oxides and iron toxicity (bronzing of leaves which is a typical nutritional disorder in wet land rice is worth mentioning).

2.13.3.Nutrient uptake under waterlogged soil conditions

Cessation of root growth and root respiration leads to a drastic drop in the uptake and transport of mineral nutrients to the shoot within a few days of waterlogging. Because the shoot dry weight continues to increase, the nutrient concentration in the shoot

declines by dilution. There is evidence that at least in several instances inhibited nutrient uptake and thus nutrient deficiency are causally involved in enhanced leaf senescence and cessation of shoot growth in plants subjected to waterlogging (Trought & Drew, 1980a).

In maize a lack of root aeration drastically lowered concentrations of nitrogen, posphorus and potassium in the shoot elongation zone and a decline in the shoot elongation growth (Marschner, 1986). In agreement with this role of nutrient deprivation in wheat, symptoms of enhanced leaf senescence induced by waterlogging could be prevented by the daily application of nitrogen (nitrate or ammonium) to the surface of a waterlogged soil where new roots were developing (Trought & Drew, 1980b). Also, in the long-term, high nitrogen fertilizer application alleviated waterlogging injury to cereal crops (Watson et al., 1976) due both to compensation for losses by de-nitrification to impaired uptake from poorly aerated soils.

Whether nitrogen deficiency is mainly caused by the de-nitrification and leaching or by a decline in absorption activity of the roots is still unknown. It is believed, however, that insufficient oxygen supply to the rhizosphere will force the plant roots to undergo anaerobic respiration which can produce only a small amount of energy. The energy thus produced is usually insufficient for normal metabolism causing many root cells to die and decay. Subsequently growth rate progressively declines, accompanied by inhibition of root growth, reduction of nutrient and water uptake and alteration in hormone balance (Singh & Ghildyal, 1980; Jackson & Drew, 1984).

The beneficial effect of additional nitrogen application on shoot growth should not be overestimated and generalized, however, because the uptake of other mineral nutrients is also impaired. Thus application of nitrogen or phosphorus alone would not be beneficial for shoot growth if the supply of other ions is limiting. Increases in the supply of all essential nutrient elements can improve plant waterlogging tolerance by reducing the rate of decline in photosynthesis, chlorophyll content, and root and shoot growth under waterlogged conditions (Huang et al., 1994a).

2.13.4. Farmers' strategy to combat waterlogging stress

2.13.4.1. Choice of cultivar

Crops vary in waterlogging tolerance or susceptibility. Among small grains, winter wheat is more tolerant than winter barley (*Hordeum vulgare* L.) or rye (*Secale cereale* L) (Bourget et al., 1966). Within species, genotypes differ in their tolerance to waterlogging for some cereal crops, including barley (Wignarajah et al., 1976), corn (Van Toai et al., 1988) and wheat (Thomson et al., 1992). Research results (Huang et al., 1994b) indicated that wheat genotypes differed not only in their responses to hypoxia in the rooting environment but also in the recovery of growth once aeration of the roots system was resumed. After aeration was resumed, shoot growth increased to the value of the control plants in the waterlogging tolerant wheat genotypes but increased only partially for the susceptible genotypes. Furthermore, the reduction in shoot growth under waterlogged conditions was most pronounced for the susceptible genotypes and least pronounced for the tolerant genotypes. Stomatal conductance also recovered within 5-10 days for the tolerant genotypes but never recovered for the susceptible genotypes. This work indicates that breeding for waterlogging tolerance could be facilitated by selecting genotypes that maintain stomatal opening under waterlogged conditions, or open their stomata after termination of waterlogging and resume seminal root growth.

Genotypic differences with regard to nutrient accumulation and distribution under waterlogged conditions have also been reported (Huang et al., 1995). Waterlogging reduced the concentrations of N, P, K, Mg and Zn in leaves and stems and increased the concentrations of those elements in the root system, the effects being greater for waterlogging-sensitive than for waterlogging-tolerant wheat genotypes.

In Ethiopian circumstances, barley production systems vary from one area to another. Population pressure, unreliable rainfall, poor soil fertility, poor drainage and frost pose serious management challenges. Accordingly, farmers have devised strategies to optimize the ever shrinking land resources. The strategies include selecting crops and/or cultivars to suit different soil types and drainage conditions; and selecting crops, seasons (*meher, belg*, irrigated,) and selecting locations with a low incidence of frost.

Based on these management strategies, five barley production systems are recognized (Yirga et al., 1998):

1) short rainy season (*belg)* barley production system (Figure 2.2)
2) early (short cycle) barley production system
3) late (long cycle) barley production system
4) residual barley production system and
5) soil burning (*guie)* barley production system (Figures 2.3)

The importance of each system varies from locality to locality and it is not uncommon to find diverse farmers' barley cultivars grown in each production system. For instance, in north-, west-, and northwest Shewa 7, 10 and 15 barley cultivars, respectively, were reported to be grown (Yirga et al., 1998). Farmers are indifferent to spatially widely adapted cultivars, but are interested in cultivars specifically adapted to their conditions, needs and uses and which have a high degree of stability over time. Accordingly cultivars for the known production systems are selected based on their known merits by farmers in the respective localities or production systems. Under the *guie* barley production system farmers prefer Mage to other cultivars because of its relative tolerance to waterlogging stress. Accordingly, they grow other cultivars on well-drained soils or near homesteads.

Figure 2.2. Short season (*belg*) barley production in Ankober district, a typical high altitude area with high rainfall.

That is why in many areas of the tropics and sub-tropics, especially in areas with marginal conditions and resource poor farmers, that we see landraces still being an integral part of the farming system. The non-adoption of modern varieties and continued use of traditional landraces by the resource poor farmers is often explained by agricultural scientists as being due to the conservative attitude of the resource poor farmers, and the inability to obtain improved material and external inputs. However, in specific instances landraces may, in a variety of cropping systems, provide better adaptation and more sustainable yield security than do modern varieties. Landraces, also called farmers' varieties, old cultivars, or primitive cultivars (Ceccarelli & Grando, 1996) are the backbone of agricultural systems in many developing countries mainly in marginal environments because in such environments the use of varieties bred for

favorable growing conditions has become difficult. While the importance of local specificity or the value of local adaptation of landraces is appreciated, environmental adaptation to marginal environments tends to be more complex and the genetic basis of the level of adaptation of local materials is also not well understood thus complicating improvement through formal plant breeding.

2.13.4.2. Soil burning ("guie")

Traditional land management systems in Ethiopia, which is mainly practiced in some parts of the central highlands, involves soil burning (*guie*) unlike vegetation burning, or simply soil drying which has been known to most ancient agriculturalists. Soil burning (*guie*) is a cultural practice where soil material is piled without additional vegetation. Such system of soil burning which is locally known as *guie* (derived from the local word "gaye", meaning burned) is practiced by farmers whenever they bring a piece of land with heavy clay, which is left fallow for about eight to 15 years, into cultivation (IAR, 1979). The fallow period is in fact reduced to five to seven years this time because of increasing population pressure. At the end of the fallow period, the land is plowed repeatedly in a criss-cross direction with a local mould board type of plough called *maresha* which is pulled by a pair of oxen. Ploughing starts at the end of the main rainy season (in October) and lasts until April. After the soil and sods are well dried (in May), they are finely crushed with hoes and then piled in random mounds in the field (Figure 2.3a). After the soil is piled, it is opened at one side and a small amount of burning cow-dung is placed in the hole. The fire is then covered with soil and the mounds are left to burn slowly for about a week or more. At the time of planting, the cool *guied* mounds are uniformly distributed over the field with a shovel or spade and mixed with the rest of the soil mass where barley is sown and plowed under (Figure 2.3a, b & c). As a result of such practice, yield of the first harvest from *guie* soil is doubled or tripled. However, in the following two to three years, after *guie*, the fertility of the soil declines sharply and yield of barley decreases drastically. Usually on the third year after *guie*, the field is turned again into fallow and left idle until the next burning cycle.

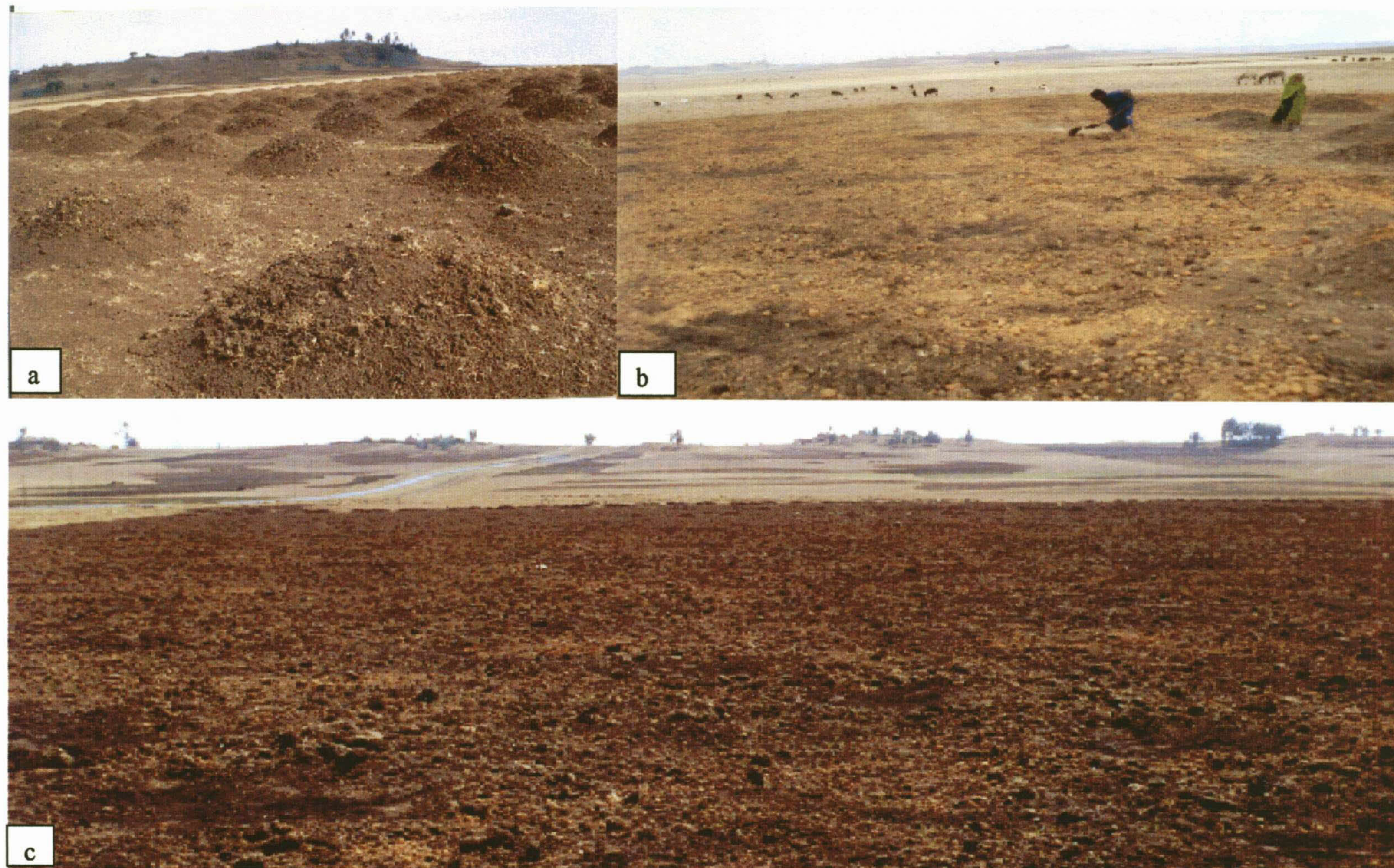

Figure 2.3. Soil burning (guie) barley production practice in north Shewa, Ethiopia: a=soil piled for burning; b=spreading the soil after guie and c=guie field planted with barley.

CHAPTER III

PHENOTYPIC VARIATION AND GENETIC RELATIONSHIPS AMONG BARLEY (*Hordeum vulgare* L.) LANDRACES FROM NORTH SHEWA IN ETHIOPIA

3.1. Abstract

There is a lack of information on genetic variability and relationships among Ethiopian barley landraces of particular environments. An experiment was conducted in a greenhouse to estimate the level of morphological variability and genetic distances within and among 44 barley landraces from north Shewa in Ethiopia. Four qualitative (spike type, kernel color, caryopsis type and spike density) and six quantitative morphological characters (spike length, number of seeds spike^{-1}, grain yield spike^{-1}, days to heading, days to maturity and plant height) were recorded from 15 plants representing each of the landraces except two represented by 10 plants, and eight by 14 plants. Data were subjected to analysis to estimate phenotypic diversity within landraces and genetic distances among them. Results indicated that morphological characters differed in amount of variation between landraces. 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'=0.95 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, 0.92, 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 but it was very high among landraces (H'=0.76). White kernel color was predominant over the others. Mean diversity index pooled over all characters ($\overline{H}$) ranged from 0.12$\pm$0.08 to 0.57$\pm$0.11, and 11 landraces had mean diversity larger than 0.50. Analysis of variance for the quantitative characters revealed that differences among landraces within localities were highly significant for all characters except for landraces from Ankober-Mezezo locality. Significant differences among localities were observed largely due to number of seeds per spike and grain yield per spike. Principal component analysis revealed that the first four principal components (PC) contributed

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81.57 % of the variability among the landraces. Number of seeds spike^{-1}, grain yield spike^{-1}, spike type and spike length contributed more to PC1, and caryopsis type, plant height, spike length and spike density to PC2. Hierarchical cluster analysis of the morphological characters resulted in 10 distinct groups of landraces with genetic distance among landraces ranging from 0.17 to 4.36.

3.2. Introduction

Cultivated barley in Ethiopia is morphologically diverse with large variation in spike type, seed color, spike density, and other phenological characters (Negassa, 1985; Asfaw, 1988, 1989b,c; Engels, 1991; Demissie & Bjornstad, 1996). This diversity reflects the wide range of ecological and human influences under which the crop has evolved. Studies done to detect genetic variability in Ethiopian barley landraces, however, involved samples from different parts of the country. Ethiopia is a country with diverse ecologies and production systems endowed with ample genetic resources including barley.

Farmers, through centuries of experience, have identified different landrace cultivars for each system of production and these cultivars are recognized by different local names. The lack of study of these landraces adapted to specific ecological conditions and utilized in the national breeding programme has been a concern of researchers, agricultural development workers and decision makers. The use of local landraces adapted to specific environments is still a topic of discussion at different forums because breeding for wider adaptation did not lead to desired objectives, especially in a country like Ethiopia where the diversity in barley growing environments is tremendous. There are still limitations to providing improved varieties adapted to specific environments and landraces are almost the sole sources of seeds for the farmers as in the case of north Shewa.

Early assessment studies of genetic variability in Ethiopian barley are acknowledged in generating information but they hardly reflect those of farmers' cultivars and collections within specific domains of production. An ethnobotanic study by Asfaw (1989c) on barley landraces from west Shewa can be cited as a pioneer in this regard but it lacks the

inclusion of quantitative traits which are important because they determine the economic use of the germplasm. In this study, farmers' cultivars grown commonly in north Shewa and landrace collections from environments where these cultivars are grown were considered to assess the level of phenotypic variability and understand the extent of genetic relatedness among the landraces to facilitate their use in the regional breeding program.

3.3. Materials and methods

3.3.1. Estimation of diversity indices

The materials consisted of a total of 44 landraces (14 dominant farmers' barley cultivars from north Shewa) and 30 accessions collected by Biodiversity Conservation and Research Institute, Ethiopia from localities where the dominant farmers' cultivars are being grown (Table 3.1). The materials were grown 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 genotypes per accession or farmers' cultivars. Only two landraces were represented by 10 genotypes 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; spike length; number of seeds per spike and grain yield per spike.

The phenotypic frequencies of the characters were analyzed by the Shannon-Weaver index to estimate the diversity of each character. The Shannon-Weaver diversity index (H') as described by Jain (1975) and Negassa (1985) is given as:

$H' = -\sum_{i=1}^{n} Pi \log_e Pi$ or sometimes used as $H' = -\sum_{i=1}^{n} Pi \ln Pi$ (Demissie & Bjornstad, 1996).

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 trait. H' was determined for individual characters. Each value of H' was divided by

its maximum value, $\log_e n$ or $\ln(n)$ in order to keep it in the range of 0-1. The average diversity ($\overline{H}'$) over K traits was estimated as $\overline{H}' = \sum H'/K$. Chi-square ($\chi^2$) analysis was performed to test deviations of each trait from the expectation according to Snedecor (1956).

Table 3.1. List of farmers' cultivars and accessions from north Shewa for variability study

No.	Cultivar/accession	Locality	No.	Cultivar/accession	Locality
1	Acc.296	G.Jarso	23	Acc.3676	Kuyu
2	Acc.653	G.Jarso	24	Acc.4319	Kuyu
3	Acc.659	G.Jarso	25	Acc.4320	Kuyu
4	Acc.1551	G.Jarso	26	Acc.4601	Kuyu
5	Acc.1552	G.Jarso	27	Mage	Kimbibit
6	Acc.1570	G.Jarso	28	Kessele	Kimbibit
7	Acc.1814	G.Jarso	29	Tikur Gebs	Ankober-Mezezo
8	Acc.1822	G.Jarso	30	Feres Gama	Kimbibit
9	Acc.3679	G.Jarso	31	Bukura	Kimbibit
10	Acc.4959	G.Jarso	32	Feleme	Kimbibit
11	Acc.4964	G.Jarso	33	Netch Gebs	Kimbibit
12	Acc.4970	G.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 Libanose	39	Acc.1609	Kimbibit
18	Acc.987	Debre Libanos e	40	Netch Ferke	Ankober-Mezezo
19	Acc.4993	Debre Libanose	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

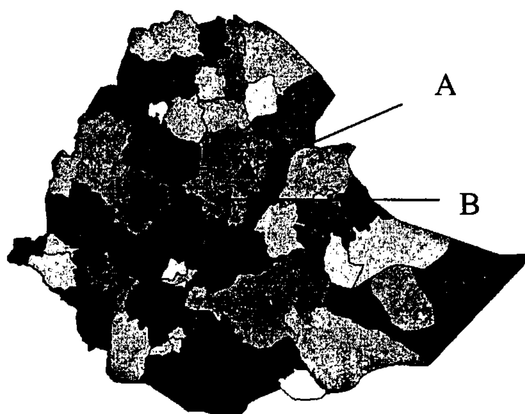

Figure 3.1. Map of Ethiopia showing north Shewa (A), Amhara region, and north Shewa (B) Oromya region, from where the landraces were collected (arrows indicate the two adminstrative zones).

3.3.2. Cluster analysis

Landraces with different spike types were split into their component spikes and cluster analysis was performed on data for morphological characters. Principal component analysis (PCA) was used as a data reduction tool to summarize the information from morphological data so that the influence of outliers on the clustering results is reduced. Hence, the 10 original morphological variables were reduced to nine independent linear combinations, principal component of the variables, with cumulative eigenvalues of 99 % because the factor with the largest eigenvalue has the most variance, down to the factors with small or negative eigenvalues that are usually omitted from solutions (Van Beuninger & Bush, 1997). Afifi & Clark (1996) claimed that eigenvalues represent variance and because the variance that each standardized variable contributes to a principal component extraction is one, a component with eigenvalues less than one is not as important (from a variance perspective) as an observed variable. Thus the most informative principal component is the first with the highest variance and the least informative is the last (a variable with zero variance) which does not distinguish between the members of the population. In this study, however, variables with cumulative eigenvalues of 99 % were taken according to Van Beuninger & Bush (1997) because cluster analysis based on these variables gave the highest cophenetic correlation coefficient.

Means of each were standardized prior to principal component analysis as suggested by Ruiz et al. (1997) to avoid the effect due to difference in scale. Standardization is achieved by subtracting from each observation the mean value of the character and subsequently dividing it by its respective standard deviation (Ruiz et al., 1997; Upadhyaya et al., 2002). This resulted in standardized values for each character with average zero and standard deviation of one or less. Ordinal variables were transformed in normal scores for spike types (1=two-rowed, 2=irregular, and 3=six-rowed); for kernel color (1=white, 2=purple, 3=black, and 4=gray); and for spike density (3=lax, 5=intermediate and 7=dense). It is assumed that transformation of ordinal variables tends to obscure clustering but it is preferred to simply assign artificial class codes (Zewdie & Zeven, 1997).

The nine principal variables were used as the input for cluster analysis. Several cluster algorithms were tried; however, Unweighted Pair Group Method of Analysis (UPGMA)

The nine principal variables were used as the input for cluster analysis. Several cluster algorithms were tried; however, Unweighted Pair Group Method of Analysis (UPGMA) of NCSS-2000 computer program appeared to give the most satisfactory clustering result with most cultivars included in clusters of similar size. A dendrogram was constructed with Euclidian distance type and average absolute deviation was used for scaling. Within cluster means and standard deviation for the quantitative characters were also computed for ease of interpretation.

3.4. Results and discussion

3.4.1. Frequencies of phenotypic characters

The percentage frequencies of the phenotypic classes of each character in the nine localities are given in Table 3.2. Individual characters differed in their patterns of distribution and amount of variation. The predominant phenotypic class in all localities is the six-row type. The highest frequencies of the six-row type were recorded for Ankober-Mezezo (85%), Kimbibit (79%), and Were Jarso (74 %) (Table 3.3). The two-row types were dominant in Wuchale (53 %) while it was absent at Ankober-Mezezo, Debre Libanose, and Were Jarso localities. A larger proportion of the irregular types occurred in Debre Libanose (71 %) followed by Kuyu (50 %) and Degem (46 %). Most landraces displayed white kernel color(able 3.2). Purple and black kernel colors were rare compared with the white and gray ones. However, purple kernel was frequently observed (34%) from populations at Kuyu. The former two combined together across localities comprised 24 % where as the white and gray types constitute 63 and 13 %, respectively (Table not indicated). The four seed color groups were found in similar proportions at Wuchale and the white and black kernel colors at Were Jarso. Landraces from Kimbibit, Ankober-Mezerzo, Girar Jarso and Wuchale predominantly displayed lax type of spike density. Eighty percent of the populations from Debre Libanose and 50 % from Were Jarso have intermediate and dense type of spikes, respectively (Table 3.2). The lax and intermediate spike densities prevailed in almost equal proportions at Kuyu and Degem (Table 3.3).

The two-rowed types had longer spike, 6 to 11 cm, than their corresponding six-row and irregular spike types but had less seeds per spike owing to their longer rachis inter-

nodes and spaced seed setting along the entire spike. The six-rowed and irregular barley types had comparable spike length 3-10 cm and 5-10 cm, respectively with a mean of nearly 7 cm. The six-rowed types retained on average 45 seeds per spike compared to 21 and 24 seeds per spike of the two-rowed and irregular types, respectively. Similarly, grain yield per spike for the six-rowed, irregular and two-rowed spike types was on average 2.19, 1.83, and 1.18 g, respectively. Accordingly, localities with the highest frequency of genotypes with the six- rowed spike types (Kimbibit, Ankober-Mezezo, and Girar Jarso) showed the highest number of seeds, greater than 40 seeds per spike, and Wuchale with the highest frequency of genotypes with the two-rowed spike types displayed less than 30 seeds per spike. The intermediate spike /irregular types which are predominantly observed at Debre Libanose and Degem have number of seeds per spike intermediate between the two-rowed and six-rowed spike types. The irregular spike types from Kuyu are exceptional in this regard in that despite 50 % of the genotypes sampled from populations at this locality are irregular, number of seeds per spike is not intermediate between the two-rowed and six-rowed types. Ahokas & Poukkula (1999) found the highest yield from six-rowed barley types which is in line with this result. It has been observed that six-rowed landraces with the highest yield showed the lowest grain weight implying a negative correlation between yield and grain mass among landraces.

All landraces except 4601 are monomorphic for caryopsis type (covered or naked kernel). Except for three landraces (4601 from Kuyu, and 4959 and 4964 from Girar Jarso) all have covered caryopsis. The naked phenotypic class was observed only at two localities (Girar Jarso and Kuyu) where 75 % of the total naked types accounted to the former locality. This can also be clearly seen from the χ^2 analysis in which all localities, but Girar Jarso, showed a non significant deviation from the expected values for caryopsis type (Table 3.3). The significant deviation from the expected distribution of caryopsis type at Girar Jarso can be attributed to the abundance of barley types with naked kernels unlike the lack of it at the other localities.

Table3.2. Percentage of phenotypic classes for qualitative and quantitative traits within 44 landraces (including farmers cultivars) evaluated in the greenhouse in 2000.

Landraces	N	Row type			Kernel color				Spike density			Days to maturity			Plant height (cm)			Number of seeds/spike		
		6R	2R	Irr	white	black	gray	purple	dense	lax	intermediate	<100	100-110	>110	<60	60-75	>75	<30	30-40	>40
144	14	100	0	0	100	0	0	0	0	79	21	0	50	50	7	21	72	14	14	72
1017	15	53	0	47	0	0	100	0	0	0	100	0	80	0	47	53	0	13	40	47
1609	15	80	0	20	100	0	0	0	0	73	27	20	40	0	7	93	0	0	13	87
3395	15	100	0	0	100	0	0	0	0	93	7	60	67	33	13	40	47	20	13	67
Bukura	10	70	0	30	100	0	0	0	30	0	70	0	60	20	0	70	30	0	30	70
Demoye	15	87	0	13	0	0	0	100	0	93	7	20	93	0	7	33	60	0	7	93
Feres Gama	15	100	0	0	100	0	0	0	6	67	27	7	40	60	0	33	67	7	13	80
Kessele	12	100	0	0	0	100	0	0	0	0	100	0	67	0	0	25	75	0	25	75
Mage	15	0	7	93	7	0	93	0	0	100	0	33	67	33	0	47	53	93	7	0
Nech Gebs	15	100	0	0	100	0	0	0	0	85	15	0	40	60	0	60	40	7	33	60
Feleme	14	100	0	0	100	0	0	0	0	71	29	0	93	7	21	50	29	14	14	72
Tikur Gebs	15	87	0	13	0	93	7	0	0	0	100	27	53	20	0	47	53	7	53	40
Key Ferke	15	93	0	7	100	0	0	0	0	100	0	0	100	0	0	47	53	0	0	100
Nech Ferke	14	60	0	40	100	0	0	0	14	33	53	50	50	0	14	64	21	7	43	50
Yeferenge Gebs	15	100	0	0	100	0	0	0	0	93	7	0	100	0	0	80	20	13	27	60
296	15	13	0	87	100	0	0	0	0	33	67	27	60	13	20	33	47	7	60	33
653	14	100	0	0	100	0	0	0	0	1	99	72	14	14	7	43	50	7	28	65
659	15	0	100	0	0	0	100	0	0	100	0	33	67	0	0	80	20	100	0	0
1551	15	0	0	100	100	0	0	0	0	53	47	33	60	7	0	13	87	27	20	53
1552	15	0	100	0	0	0	0	100	0	87	13	73	27	0	0	100	0	100	0	0
1570	10	100	0	0	100	0	0	0	0	40	60	0	80	20	0	40	60	0	40	60
1814	14	79	0	21	100	0	0	0	57	0	43	7	79	14	43	36	21	21	71	8
1822	14	100	0	0	100	0	0	0	0	93	7	0	38	64	14	72	14	21	21	58
3679	15	20	27	53	64	0	0	36	0	100	0	0	60	40	7	73	20	60	7	33
4959	15	100	0	0	0	100	0	0	100	0	0	53	47	0	67	33	0	0	40	60
4964	15	100	0	0	20	0	0	80	100	0	0	33	67	0	87	13	0	7	20	73
4970	15	40	0	60	100	0	0	0	0	13	87	33	67	0	0	73	27	13	27	60
973	15	0	100	0	100	0	0	0	0	100	0	0	100	0	0	33	67	100	0	0
976	14	0	0	100	0	0	100	0	0	36	64	43	36	21	7	14	79	14	29	57
1182	15	74	13	13	0	100	0	0	87	13	0	33	67	0	7	80	13	40	13	47
2812	15	0	100	0	0	0	0	100	0	100	0	87	13	0	40	47	13	100	0	0
984	15	13	0	87	100	0	0	0	0	47	53	0	73	27	0	20	80	27	13	60
987	15	33	0	67	100	0	0	0	0	13	87	20	80	0	7	13	80	13	74	13
4993	15	40	0	60	100	0	0	0	0	0	100	67	33	0	33	67	0	0	60	40
3151	15	40	47	13	100	0	0	0	0	60	40	27	73	0	13	47	40	33	0	67
3676	14	0	0	100	86	0	7	7	29	57	14	0	93	7	7	29	67	22	64	14
4319	15	0	0	100	80	0	13	7	0	53	47	0	33	67	0	60	40	60	27	13
4320	15	0	0	100	13	0	20	67	0	60	40	0	67	33	0	7	93	13	40	47
4601	14	100	0	0	12	0	0	88	0	0	100	0	100	0	57	43	0	7	7	86
1153	14	100	0	0	0	100	0	0	100	0	0	14	86	0	7	43	50	0	0	100
1156	15	47	0	53	100	0	0	0	0	40	60	7	93	0	0	20	80	0	40	60
Haddo	11	45	36	19	100	0	0	0	0	82	18	0	18	82	0	73	27	64	27	9
Tolese	15	7	20	73	100	0	0	0	0	20	80	53	27	20	13	67	20	60	33	7
Baleme	11	0	64	36	0	0	100	0	0	100	0	0	45	55	10	45	45	100	0	0
Total	**630**	**55**	**14**	**31**	**63**	**11**	**12**	**13**	**12**	**49**	**39**	**21**	**62**	**17**	**12**	**47**	**41**	**27**	**25**	**48**

Table 3.3. Percentage of phenotypic classes and chi-square (χ^2) values for each locality for four qualitative morphological traits

Locality	*N*	*Spike type*				*Kernel color*					*Spike density*				*Caryopsis type*		
		6R	*2R*	*Irr*	χ^2	*White*	*Black*	*Gray*	*Purple*	χ^2	*Dense*	*Lax*	*Intermediate*	$\chi 2$	*C*	*N*	χ^2
Kimbibit	155	79	1	20	44.15***	65	9	18	8	8.39	3	60	37	3.95	100	0	10.53
Girar Jarso	172	54	18	28	4.12	64	8	10	18	5.57	22	43	35	3.25	83	17	35.47*
Wuchale	59	20	53	27	81.59***	25	25	25	25	37.01***	22	62	16	21.96**	100	0	4.00
Kuyu	73	39	11	50	12.28	58	0	8	34	31.45***	6	46	48	2.08	86	14	6.61
Debre Libanose	45	29	0	71	33.82***	100	0	0	0	25.96**	0	20	80	28.47**	100	0	3.05
Were Jarso	29	74	0	26	5.93	50	50	0	0	43.15***	50	20	30	19.79*	100	0	1.96
Ankober-Mezezo	60	87	0	15	19.29*	75	23	2	0	22.27***	3	57	40	4.50	100	0	4.00
Degem	26	26	28	46	10.55	100	0	0	0	14.99	0	51	49	3.48	100	0	1.76
Welmera	11	45	19	36	25.42**	0	0	100	0	78.62***	12	49	36	11.28	100	0	0.75

Note: *, **, and *** are significant at P = 0.05, 0.01 and 0.001, respectively.N= number of genotypes; 6R = 6-rowed; irr = irregular; 2R = 2-rowed

The Chi square (χ^2) analysis revealed that all localities except Kimbibit, Girar Jarso and Degem demonstrated significant deviation from the expected distribution of kernel color (Table 3.3). At Kimbibit, Girar Jarso and Degem, however, the expected distribution of the trait was observed. Localities did not show significant difference for spike density except Wuchale, Debre Libanose, Girar Jarso and Were Jarso.

3.4.2. Estimates of diversity index

Estimates of diversity (Shannon Weaver diversity index) for individual characters, populations and localities are shown in Tables 3.4 and 3.5. Individual traits showed a different pattern of variation. Among the qualitative traits, variation for spike types (six-rowed, two-rowed or irregular forms) was high in many landraces. Fifteen landraces (34 %) showed values in the range of H'=0.55 to H' = 0.95 for this trait. A highly diverse population for this trait was Haddo from Degem locality with an estimated diversity value of H'=0.95 followed by 3679 from Girar Jarso with H' = 0.92. The overall diversity for this trait pooled over localities is also very high (H' = 0.89). Degem, Wuchale, Girar Jarso and Kuyu are highly diverse for this trait, H' = 0.96, 0.92, 0.91, and 0.79, respectively (Table 3.5). Diversity for this trait in Ankober-Mezezo locality is very low (H' = 0.39) which is in agreement with reports of Asfaw (1988) where he found quite small numbers of morphotypes per field sampled randomly from similar areas. He detected 83 % six-rowed (including intermediates) and 17 % two-rowed barley types from samples collected in barley fields. This proportion is compatible with results from this study and that of Demissie & Bjornstad (1996) for Ethiopian barley germplasm. The corresponding figures for six-rowed, two-rowed and irregular spike types in this study are 55 %, 14 % and 31 %, respectively (Table 3.2). Many authors (Negassa, 1985; Engels, 1991; Demissie &Bjornstad, 1996) noted that the two-rowed spike types concentrated much in barley growing areas with moisture stress (eg. North of Ethiopia, Tigray) and the alternate phenotypes were found in abundance as one go from north to south of Ethiopia. The relative abundance of the six-rowed barley types in north Shewa, an area receiving a high amount of rainfall, is therefore not surprising.

Within landraces, variability for kernel color was generally very low except in those from Kuyu. (acc.4320, acc.4319, and acc.3676) and acc.3679 from Girar Jarso as presented in Table 3.4 whereas variability for kernel color among landraces was very

high (H'= 0.76).The abundance of white kernel color in this study is in line with an investigation by Demissie and Bjornstad (1996) on Ethiopian barley landraces. However, their study revealed that the black kernel color stands next in frequency while this study showed that gray kernel color stood next to white.

Caryopsis type (covered or naked grain) was the least diverse character observed in this investigation supporting a previous study on Ethiopian barley landraces by Negassa (1985) and Demissie & Bjornstad (1996). Negassa (1985) found naked phenotypes only in Gamo Gofa and Shewa administrative regions indicating that naked barley types are restricted in occurrence. This study reported naked types predominantly with white kernel color. In this study kernel color of the naked barley types was predominantly purple and black with rare white types. Vitreous to starchy endosperm was found in naked lines from Ethiopia and this indicated that the possibility exists in increasing the gluten content of barley to increase barley quality (Negassa, 1985). However, farmers in Ethiopia do not commonly grow these barley types because they have low yield potential and small grain size to their alternate covered types. The breeding program also did not give emphasis to these barley types and it may be important to assess merits of naked barley types and utilize them, at least in the long term, as donors of genes for improved nutritional quality.

In general, the mean diversity index (H') values pooled over traits of barley landraces from north Shewa ranged from 0.12 to 0.57 (Table 3.4). Only 11 landraces had a mean diversity index (H') pooled over all traits, greater than 0.50. The highest mean diversity (H'=0.57) was shown by landrace 4320 from Kuyu followed by Tolese and 4319 with mean H' values of 0.56 and 0.55, respectively. Among the farmers' cultivars only Nech Ferke and Tikur Gebs from Ankober-Mezezo locality showed higher within landrace variation with H' values of 0.54 and 0.53, respectively. The lowest H' value (H' = 0.12) was recorded for landrace 973 at Wuchale locality. Landraces from Girar Jarso, Wuchale, Kuyu, Were Jarso and Kimbibit differed significantly in spike type and kernel color (Table 3.4).

Many workers (Qualset, 1975; Negassa, 1985; Asfaw, 1988, 1989b,c; Demissie & Bjornstad, 1996) have reported the level of diversity in Ethiopian barley landraces but only Engels (1991) made estimates of diversity for quantitative traits. In this study,

spike length and number of seeds per spike were included in addition to what had been used by Engels (1991) to give a more general assessment of diversity for quantitative traits in barley landraces from north Shewa. From a plant breeding point of view, the use of such quantitative characters which are scaled in an arbitrary way are useful because they are of more interest to the plant breeder than the discrete or qualitative characters. Estimated diversity indices for quantitative traits were generally high. The assertion from this study was similar to that of Engels (1991) except that values of diversity indices in his study were very high. Nonetheless, Engels (1991) remarked that in such studies the number of classes per character were set arbitrarily and consequently this will influence the magnitude of the diversity index. Hence a comparison of the diversity indices for the quantitative characters with other studies may be meaningless.

Table 3.4. Estimates of diversity indices within 44 barley landraces from different localities of north Shewa, Ethiopia.

Landraces	N	Spike type	Kernel color	Spike density	Caryopsis type	Spike length	PLH	DMA	NS/SP	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 Gama	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 Ferke	14	0.61	0.00	0.97	0.00	0.72	0.63	0.63	0.81	0.54±0.13
Yeferenge Gebs	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 Shewa	**630**	**0.89**	**0.76**	**0.88**	**0.33**	**0.73**	**0.89**	**0.84**	**0.96**	**0.76±0.09**

Table 3.5. Mean diversity index (H') of each character for genotypes from various localities and the overall populations from north Shewa, Ethiopia

Locality	N	Spike type	Kernel color	Spike density	Caryopsis type	Spike length	PLH	DMA	NS/SP	$\bar{H}$±S.E
Kimbibit	155	0.50	0.58	0.72	0.00	0.66	0.58	0.82	0.79	0.58±0.09
Ankober-Mezezo	59	0.39	0.45	0.73	0.00	0.83	0.74	0.62	0.77	0.57±0.10
Girar Jarso	172	0.91	0.71	0.96	0.65	0.55	0.93	0.87	0.98	0.82±0.09
Wuchale	59	0.92	1.00	0.84	0.00	0.62	0.91	0.76	0.80	0.73±0.09
Debre Libanose	45	0.39	0.00	0.46	0.00	0.43	0.88	0.80	0.90	0.50±0.10
Kuyu	73	0.79	0.64	0.80	0.29	0.77	0.91	0.65	0.97	0.74±0.09
Were Jarso	29	0.52	0.50	0.94	0.00	1.00	0.67	0.30	0.46	0.55±0.12
Degem	26	0.96	0.00	0.63	0.00	0.77	0.66	0.93	0.78	0.59±0.12
Welmera	11	0.59	0.00	0.00	0.00	0.94	0.86	0.63	0.25	0.41±0.14
North Shewa	**630**	**0.89**	**0.76**	**0.88**	**0.33**	**0.73**	**0.89**	**0.90**	**0.98**	**0.76±0.09**

N=total number of genotypes sampled

Using the raw data of the 44 landraces from the three replications, analysis of variance for the six quantitative characters was done to assess differences among landraces within localities and possible differentiation among localities. Differences among landraces within localities are presented in Table 3.6. Were Jarso, Kuyu, Debre Libanose and Welmera were treated together with Wuchale–Degem in the analysis because number of landraces from these localities are very few and the error degrees of freedom will be low to treat them separately. Differences among landraces within localities were highly significant for all characters except with landraces from the Ankober-Mezezo barley production area. Landraces from this locality were not apparently different in plant height, spike length and grain yield per spike. The range of values for the different characters of landraces from this area were also narrow in view of landraces from other localities which could be because of the small number of samples. The presence of intermediate and lax spike types in this group of landraces brought differences in number of seeds and grain yield per spike despite lack of significant differences in their spike length. These landraces appeared to have very good spike length, number of seeds per spike, and grain yield per spike compared to landraces from the other localities.

The analysis of variance also suggested significant differentiation among localities largely due to number of seeds per spike and grain yield per spike but not for days to maturity, plant height and spike length (Table 3.7). The Duncan's multiple range test for

locality means indicated that number of seeds per spike and grain yield per spike of landraces from Ankober-Mezezo, Kimbibit, Girar Jarso and Debre Libanose were significantly (P=0.01) different from the other localities. If such significant phenotypic differences for quantitative characters within and among landraces are assumed to be largely due to genetic effects, improvement can be achieved by selection within or among landraces. However, accurate scoring of flowering and maturity is difficult in part because different plants in the same plot do not flower or reach maturity at the same time because of several factors (Rasmusson et al., 1979). Hence, it is difficult to conclude that such high magnitude in variation for quantitative characters within landraces is absolutely genetic.

Table3.6. Within locality mean square values and statistical significance for quantitative characters in barley landraces from north Shewa, Ethiopia, evaluated in the greenhouse.

Locality	DF	Days to heading	Days to maturity	Plant height	Spike length	Number of seeds per spike	Grain yield per spike
Kimbibit	18	48.000**	56.060***	104.410**	3.690*	222.810***	0.568***
Ankober –Mezezo	6	34.440*	27.780NS	34.330NS	1.000NS	101.560**	0.123NS
G.Jarso	25	44.150**	58.150***	190.630***	3.470***	243.120***	0.557***
Wuchale-Degem	28	57.740**	87.420***	187.090**	3.490***	151.890**	0.318**
Overall	83	47.340***	62.260***	154.390***	3.480***	226.380***	0.515***

DF = degrees of freedom ; NS = non significant; *, **, *** = significant at P= 0.05, 0.01, & 0.001 probability level, respectively.

Table 3.7. Means of landraces from specific localities and statistical significance for the different quantitative characters

Locality	DHE	DMA	PLH	SPL	NS/SP	GY/SP
Kimbibit	71	106	74	7.2	42a	2.11a
Ankober-Mezezo	74	104	74	7.5	44a	2.27a
Girar Jarso	73	104	70	7.4	38ab	1.91ab
Debre Libanose	70	103	76	7.6	38ab	1.95ab
Degem	74	101	70	7.6	29c	1.61b
Kuyu	71	106	74	7.9	37abc	1.86ab
Wuchale	70	102	73	7.6	30bc	1.60b
Welmera	81*	108	74	8.2	15d	0.87c

DHE & DMA = days to heading & maturity, respectively; PLH = plant height (cm); SPL= spike length; NS/SP= number of seeds per spike; GY/SP= grain yield per spike (gm). * = only Welmera significantly (P=0.05) differed from other localities. Means followed by the same letter in a column are not significantly different at P= 0.05.

3.4.3. Genetic relationships from cluster analysis

Principal component analysis (PCA) revealed that the first four components (PCs) with eigenvalues greater than one contributed 81.57 % of the variability among the landraces evaluated for 10 morphological characters (Table 3.8). Characters which contributed more to PC1 were number of seeds spike^{-1}, grain yield spike^{-1}, spike type and spike length whereas caryopsis type, plant height, spike length and spike density contributes to PC2. PC3 separated landraces on days to heading and days to maturity while PC4 separated only on kernel color.

Table 3.8.Principal components and their percentage contribution to the total variance.

Principal components	Eigenvalues	Contribution of each PC (%)	
		Individual	Cumulative
PC1	2.923	29.23	29.23
PC2	2.242	22.42	51.65
PC3	1.889	18.89	70.55
PC4	1.102	11.03	81.57
PC5	0.722	7.23	88.80
PC6	0.505	5.06	93.86
PC7	0.236	2.36	96.22
PC8	0.174	1.74	97.96
PC9	0.132	1.32	99.29
PC10	0.071	0.71	100.00

Cluster analysis based on the nine principal variables enabled grouping of the landraces into nine clusters which share some common morphological features. Splitting the landraces into their spike categories before performing the cluster analysis resulted in a very good cophenetic correlation (0.92). A cophenetic correlation value indicates a Pearson correlation between the actual distances based on particular hierarchical configuration and a value of 0.75 or above needs to be achieved in order for the clustering to be useful (Hintze et al., 1998).

Cluster I. It is the second largest cluster consisting of 11 landraces all with six-row spike types. White seed color with long spike length (except *Bukura*) resulting in a high number of seeds and grain yield per spike are characteristic features of landraces in this cluster. Some of the important local cultivars (Feres Gama, Nech Gebs, Feleme and Yeferenge Gebs*)* recognized by farmers to give very good yield were members of this cluster.

Cluster II. This cluster comprised of 10 landraces all with irregular types of spikes, except acc.296, and white seed color. They had very long spikes, had comparable days to heading and maturity as landraces in cluster I but were lower in number of seeds and grain yield per spike.

Cluster III. Both irregular and six-rowed barley landraces with white seed color, except Demoye(IR), were found in this cluster. Plant height, number of seeds per spike and grain yield per spike in these landraces are comparable to those in cluster I but they are earlier in days to heading and maturity than the irregular and six-rowed types in cluster I and II. Hence, it is possible to conclude that these landraces are clustered separately from the six-rowed and irregular types in clusters I and II mainly because of their differences in days to heading and maturity.

Cluster IV. All are six-rowed types with purple, black or white seed color, early in days to heading as those in cluster III but with a higher number of seeds per spike and grain yield per spike than all landraces in cluster I, II, and III.

Cluster V. Two farmers' cultivars (Kessele and Tikur Gebs) and acc.976 appeared in this group. They all had long spikes and a comparable number of seeds per spike and grain yield per spike to landraces in cluster I. Earliness and seed color seems distinguishing characters of landraces in this group. The former two had black seed color and the latter purple. Kessele and Tikur Gebs were collected from different agroecological zones and production systems, but they are very confusing because of similarities in seed color and maturity days. Often they are regarded as the same landraces with different names given by different ethnic groups to symbolize their seed color (Kessele in Oromic language and Tikur Gebs in Amharic).

Cluster VI. comprised of only two six-rowed landraces, very early in days to heading and maturity as in cluster III but had very short plant height, shorter spike length and had black or gray seed color.

Cluster VII to X. Landraces in cluster VII and VIII were characterized by a very low number of seeds per spike which are either two-rowed or irregular types. However, landraces in cluster VIII had very short plant height, and hence were in different cluster. The two-rowed landraces in the latter cluster compare very well to landraces in cluster VI in plant height but they were very late for days to heading and maturity. Acc.1552, acc.2812 and acc.3151(2R) are also two-rowed types but they all had a fairly high number of seeds per spike and grain yield per spike, earlier in days to heading and maturity than their corresponding two-rowed types in cluster VIII, and hence appeared separately in cluster IX. Interestingly, the cluster analysis was able to distinguish the three landraces with naked grain and put them separately as group X in the dendrogram. They had dense spikes and were shorter in plant height than any of the landraces evaluated.

Table 3.9. Clusters and morphological characters of landraces grouped in the same cluster based on data from a greenhouse experiment in 2000.

Cluster	DHE	DMA	PLH	Spike type	Caryopsis type	Kernel color	Spike length (cm)	NS/SP	GY/SP(g)
I	75±3.00	109±2.59	74±3.8	6R	C	W	6.9±0.54	43±5.42	2.27±0.16
II	74±2.02	107±3.29	78±5.70	IR	C	W	8.1±0.54	36±4.31	1.95±0.24
III	67±2.32	100±1.70	71±4.94	IR, 6R	C	W	7.5±0.62	42±5.95	2.05±0.26
IV	68±3.06	104±0.00	77±2.08	6R	C	P,B,W	8.2±0.72	54±2.89	2.87±0.16
V	72±2.64	104±2.00	80±1.15	IR, 6R	C	B,G	7.8±0.43	41±3.21	2.13±0.10
VI	66±0.00	97±2.83	66±9.19	6R	C	B,G	6.9±0.42	45±0.70	1.99±0.09
VII	79±2.75	107±3.09	74±3.59	2R,IR	C	G	8.0±0.46	19±4.69	1.05±0.17
VIII	81±0.71	112±0.71	66±1.41	2R	C	W	8.2±0.49	17±4.24	0.97±0.19
IX	66±2.08	96±3.06	65±3.00	2R	C	P,W	8.4±1.07	21±1.26	1.18±0.18
X	71±1.00	101±2.31	56±1.53	6R	N	P,B	6.7±0.21	43±1.00	1.71±0.09

DHE & DMA = days to heading and maturity, respectively; PLH = plant height; NS/SP = number of seeds per spike; GY/SP = grain yield per spike; C= covered, N = naked; W= white; B = black; P = purple and G = gray.

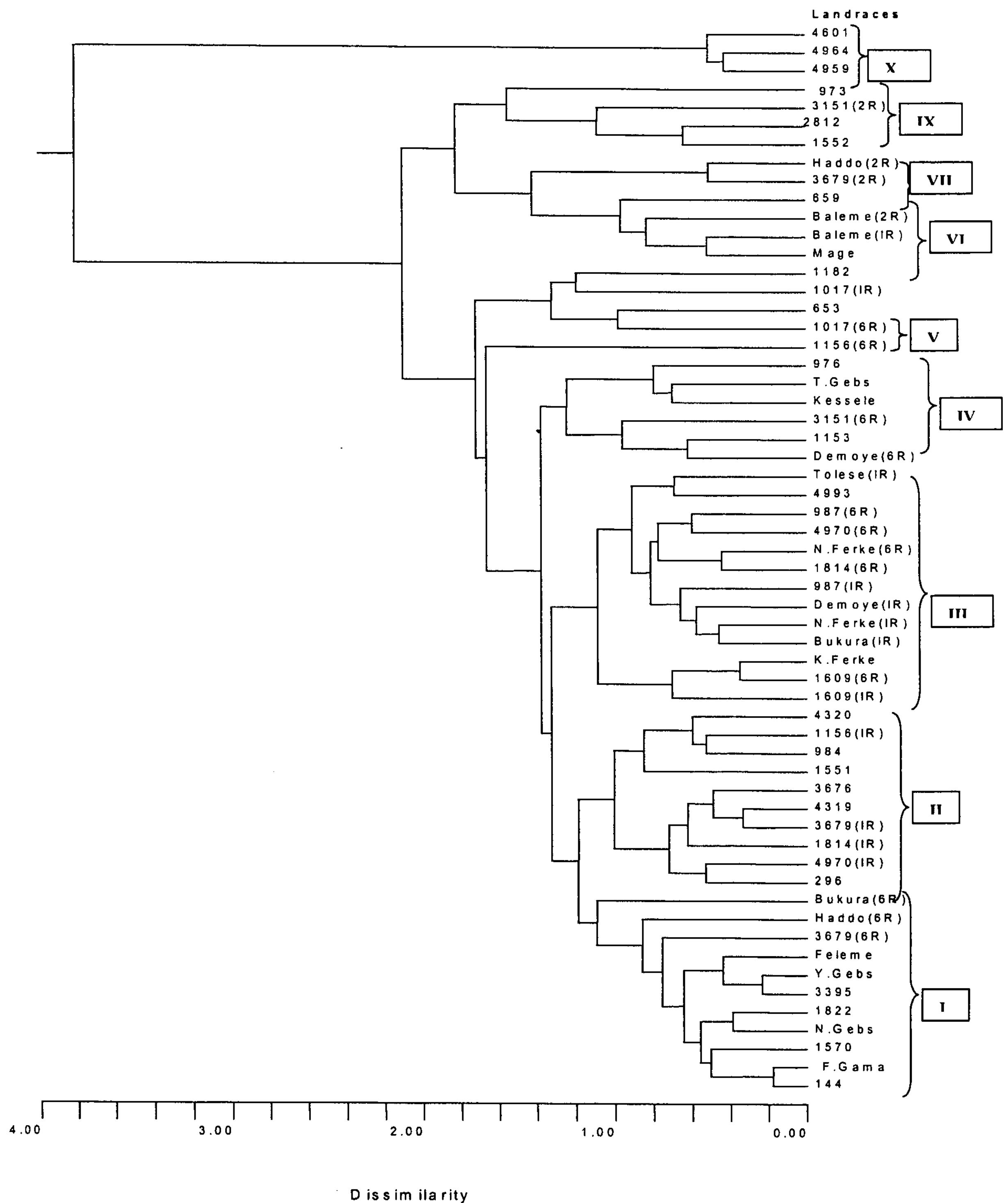

Figure 3. 1. Cluster groups based on the morphological data set from 14 farmers' cultivars and 30 accessions.

3.4.4. Conclusions

Because there is increased interest in the utilization of landraces adapted to specific environments in breeding programs, it would be necessary to assess how much variability they display without excluding the fact that variability alone can not be taken as a guarantee for improvement unless they possess traits of economic significance. Variation pooled over traits, both qualitative and quantitative, ranged from very low (H' = 0.12±0.08) in acc.973 to high ($\overline{H}'$= 0.57± 0.11) in acc.4320. Within farmers' cultivars variation pooled over traits, both qualitative and quantitative, ranged from very low (H' = 0.20±0.09) in Key Ferke to high (H' = 0.56± 0.13) in Tolese. There was no detectable variation for kernel color and caryopsis type within farmers' cultivars in general. Notable variability existed for spike type and spike density in Bukura, Nech Ferke, Haddo and Tolese, however. Feres Gama and Feleme were found to be variable in spike density only. The variability for quantitative characters is very high in many of the farmers' cultivars. The variation is less in Feleme and Key Ferke, however. Unlike farmers' cultivars, the accessions were found to be more variable in spike type and kernel color. Moreover, the mean diversity indices pooled over characters were relatively higher in the accessions than within farmers' cultivars.

Practical implications of results from this diversity study can be looked at in two ways. Firstly for qualitatively inherited morphological characters that are coded by one or a few genes, undesirable alleles can be eliminated completely. Genotypes homozygous for the desired allele can be identified phenotypically and a single step of selection for qualitative morphological characters can completely successfully remove the undesirable genotypes. Progress in improvement for quantitative characters can be achieved if selection is exercised to isolate components of spike types in some of the farmers' cultivars and landrace collections displaying variation for this character. This will ultimately bring about some improvement in economically important traits such as grain yield. Hence, Bukura, Nech Ferke, Haddo, Baleme, Tolese, acc.987, acc.4993, acc.3151, acc.4320 and acc.1156 lend themselves to selection for spike types.

The second aspect is looking for variation in the quantitative characters that are polygenic. Estimated diversity indices for the different quantitative characters have

demonstrated ample variation. In this situation the probability of finding the best genotype at all loci is extremely small because only the phenotypes can be observed and the best genotype may be overlooked even if it is in the population. For characters showing continuous variation, statistical parameters like means and variances are used to measure the response to selection. This response depends not only on the genetic variance, but also on the non-genetic variance and interaction of the genetic effects with the environment. Hence, even though the estimated diversity values within landraces for the quantitative characters indicate ample variation, it would be difficult to explicitly conclude that progress can be made through selection for these traits because of reasons explained above. Progress that may be achieved through selection for quantitative characters shall be investigated further following field experiment across locations and years. Another outcome of this study was the understanding of the genetic relationship among landraces which will give insight in which landraces to focus on for crossing based on their genetic distances and desirable agronomic features.

CHAPTER IV

GENETIC VARIABILITY IN BARLEY (*Hordeum vulgare* L.) LANDRACES FROM NORTH SHEWA IN ETHIOPIA AS REVEALED BY SDS-PAGE OF SEED STORAGE PROTEINS

4.1. Abstract

Although early variability studies based on protein markers have increased knowledge on barley landraces of Ethiopia, it had limited practical implications from a plant breeding point of view since the evaluations were not systematic and region specific that enable the exploitation of specific adaptation of landraces. The aim of this study was to supplement variability data based on morphological descriptors and facilitate the use of germplasm within the same region and at the same time reduce the risk of losing essential adaptive characters of landraces from target environments. The study comprised 44 landraces, including 14 farmers' cultivars, each represented by 15 lines. SDS-PAGE of seed storage proteins was carried out for each landrace to estimate the level of variation within landraces. The genetic relationship between landraces was investigated based on electrophoretic banding patterns pooled from each landrace. SDS-PAGE revealed very low to high variation both within and among landraces. Although 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 within landraces ranged from 0.353 to 0.678 and 0.235 to 0.881 between landraces. Variability within most of the farmers' cultivars was generally lower than within accessions. Mean genetic distances between landraces within localities were lower (0.462 ± 0.11 to 0.615 ± 0.10) than mean genetic distances of landraces between different localities whose values ranged from 0.405 ± 0.05 to 0.758 ± 0.06. SDS-PAGE did not provide discrimination between landraces according to their origin although some landraces were clustered together according to their geographical origin. No association has been found between measures of variability based on morphological descriptors and SDS-PAGE data. Landraces, genetically variable and at the same time possessing desirable agronomic features, have been identified from which pure line selection and evaluation can be carried out in the future. Future crossing programs

between genetically distant landraces can be envisaged following evaluation of the pure lines from landraces with desirable agronomic features.

4.2. 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 provide predictive estimates of genetic variation among segregating progeny for pure line cultivar development (Manjarrez-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 (Cox & Murphy, 1990; Barbosa-Neto et al., 1996). Crosses made between genetically distant genotypes within major cluster 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 and superior recombinants 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 identifying well performing parents that have not been used in crossing programs.

In Ethiopia the development of distinct cultivars tailored to specific production environments has not been implemented as anticipated. One of the gaps is lack of assessing the level of genetic variability among adapted germplasm and thereby making crosses within such gene pool. Environmental adaptation involves many complex traits and the use of germplasm developed within the same region targeted for cultivar improvement reduces the risk of losing essential adaptive characteristics through recombination (Barrett and Kidwell, 1998).

Protein markers such as isozyme variants of esterases (Kahler & Allard, 1970; Havid & Nielson, 1977), allozymes (Bekele, 1983a) and hordein (Shewry et al., 1978a, 1978b; Doll & Brown, 1979) 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, a simple genetic control, a complex molecular basis for genetic diversity, and homologies between storage proteins that extend across taxa (Gepts, 1990). Many analytical methods have been used to characterize protein in general and seed proteins in particular. Polyacrylamide gel electrophoresis in an SDS-containing medium (SDS-PAGE) offers the advantage that all proteins are solubilized, negatively charged, and randomly coiled. In the Ethiopian circumstances, the use of biochemical criteria such as esterase and acid phosphatase (Bekele, 1983a; 1983b), flavonoids (Bekele, 1984), and hordeins (Asfaw, 1989a, 1989b; Demissie & Bjornstad, 1997) have been used for the estimation of diversity of barley landraces. Although the information generated increased knowledge on barley landraces of Ethiopia, it had limited practical implications from breeding point of view since the evaluations were not systematic and region specific. The aim of this study was to assess variation within and between barley landraces from north Shewa in Ethiopia and generate information that facilitates the efficient utilization of landraces in national or regional breeding programs. The study focused on farmers' cultivars and collections from areas where these cultivars are grown.

4.3. Materials and methods

4.3.1. Plant materials

Fourteen dominant farmers' cultivars and 30 accessions collected from localities where these cultivars are widely grown were used for this study (Table 3.1). Fifteen random spikes represented each of farmers' cultivars and accessions. A single seed from each spike was used for the analysis.

4.3.2. Storage protein extraction

Single kernel extraction was employed throughout this study. Individual seeds were ground with a mortar in a pestle and the ground kernels were transferred to 1.5ml Eppendorf tubes. Extraction buffer (500 µl, containing urea, 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. Elevated temperature (60°C) and a strong reducing agent [2 % (V/V) 2-mercaptoethanol] were necessary to extract hordein from ground whole grain. In 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; 1978a). After an hour, the tubes were taken from the water bath and centrifuged for three to four minutes at 10 000 rpm using an Eppendorf microcentrifuge. Eighty µl of the supernatant from each of the hordein extracts was transferred to a new Eppendorf tube containing 80 µl sample buffer and centrifuged for 2 minutes. The sample buffer was prepared from 1 g Tris and 90 ml 50 % n-propanol titrated to pH 8.0 with NaHCL and thereafter 40 g glycerol, 2 g SDS and 0.02 g bromophenol blue were added.

4.3.3. Preparation of the gel

A 10 % uniform separating gel was used. The gel system adapted was that described by Singh et al. (1991) with some modifications. The gel consisted of a separating and stacking gel. The separating gel was composed of 38 ml separating buffer (45.412g Tris dissolved in 460 ml distilled water, titrated to pH 8.0 to which 1g SDS was added), 28 ml separating acrylamide of 30 % Ac/1 % cross linker (38g acrylamide and 0.38g bisacrylamaide dissolved in 90ml distilled water) and 14 ml distilled water. Hundred and sixty five µl N,N,N',N-tetramethylethylenediamide (Temed) (165 µl), and 190 µl 10 % ammonium persulphate (APS) were used as catalysts.

The stacking gel was composed of 10 ml stacking buffer (6.06 g Tris dissolved in 190 ml distilled water titrated to pH 6.8 and 0.4 g SDS); 2.6 ml stacking acrylamide (35 % Ac/1.5 % cross linker which is composed of 44g acrylamide and 0.66g bisacrylamide (dissolved in 90 ml distilled water), and 7.4 ml distilled water. Temed (40 µl) and 100 µl 10 % APS

were used as catalysts. The gel cassettes and the spacers were assembled and the respective gel solutions (separating gel and stacking gel) were poured into the gel former in order. A twenty sample well former (0.75 mm perspex comb) was inserted into the stacking gel and left overnight to polymerize. Distance between the sample wells and the separating gel was maintained at about 1.0cm to 1.5cm throughout the study.

4.3.4. Electrophoresis

The next day hordein extracts from individual kernels (each 40 μl) were loaded into each sample well with a micropipette. Twenty slot gels were run with the standard 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. Composition of the cathode buffer (upper tank) used was 15.14 g Tris, 72 g glycine, and 5 g SDS made up to 500 ml with distilled water. The anode buffer (lower tank) consisted 30.28 g Tris, 800 ml distilled water which is titrated to pH 8.4. Both the cathode and anode buffers were diluted 10x before use. Two gels were run simultaneously on one Biorad 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.

4.3.5. Staining of the gel

Upon completion of each run, gels were removed from the gel cassette and put in a plastic container containing a fixing solution (50 ml glacial acetic acid, 200 ml methanol and 250 ml distilled water) for more than an hour. Then the gels were stained immediately with a mixture of 30g trichloroacetic acid dissolved in 200 ml distilled water, and 0.1 g Coomassie Brilliant Blue in 10 ml methanol. The box containing the gels was put on a shaker dancer to maintain a uniform spread of the staining solution over the gels. For convenience, the gels were stained overnight and then de-stained with distilled water.

4.3.6. Reading the gel

By comparing the Clipper banding patterns across the gel, an indication of the uniformity of conditions across the gel was ensured. The gels were scanned with a Biorad scanner connected to a computer program. The scanned images were saved and the migration distances of protein bands were obtained from densitometry scans using Molecular Analyst Finger Printing system (Biorad Labs. Hercules, C.A) that gave a density *versus* distance plot. This plot gave quantitative information about the density of each band and a measure of how far down the gel each protein band had run. Only bands with an intensity of ten and above were accepted for reading.

4.3.7. Statistical analysis

Bands were scored as absence (0) and presence (1) based on their migration distances and entered for each landrace separately as a binary data matrix for statistical analysis. Bands that are present across all lines in a landrace were excluded from statistical analysis while bands absent in at least one line were recorded. Genetic distance was calculated using NCSS 2000 software applying pair-wise comparison using the formula $1\text{-}F=1\text{-}[2n_{xy}/(n_x+n_y)]$ 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.

4.4. Results and discussion

4.4.1. Genetic variability within landraces

Range, mean and frequency of genetic distance values from SDS-PAGE of storage proteins among lines sampled from each landrace are shown in Table 4.1. Assessment of variability 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. Banding patterns of two landraces with intermediate and low

genetic variability are presented in Figure 4.1. 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 $\geq$ 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 in which as high as 46 % of all possible comparisons between samples in the landraces were identical (Table 4.1). 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 hordein variability did not reveal high differentiation among lines. On the other hand SDS-PAGE uncovered components within landraces different from each other which otherwise are 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 in banding patterns confirming that they comprised different genotypes. 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 (Table 4.1).

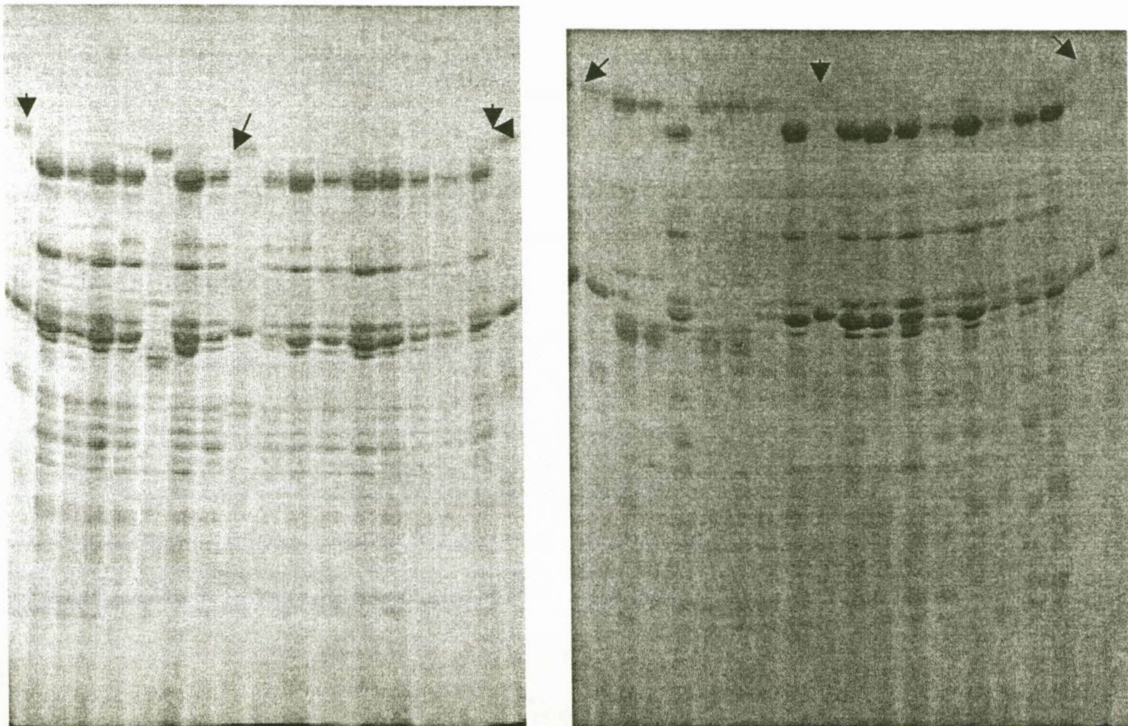

Fig.4.1. Banding patterns from SDS-PAGE of storage proteins of Nech Ferke (a) with intermediate (0.547) and Demoye (b) with low (0.356) mean genetic distance between lines. Arrows indicate lanes of the reference cultivar Clipper.

Differences in band intensities of samples within the same landrace were observed (Figure 4.1) and this could be an indication of differences in hordein contents among the different lines. Those with weaker band intensity perhaps have higher lysine content than those with dense bands because the presence of an inverse relationship between hordein and lysine content has been reported (Doll &Brown 1979; Shewry & Miflin, 1982). Such variation in band intensity of samples from the same landrace cannot be attributed to differences in gel staining period as all samples from one landrace were run and stained at the same time and duration. Hence, the above assertion on the relation between intensity and amount of hordein may hold true. Asfaw (1989a), however, suggested that samples in which the bands were less intense should undergo further tests to see their lysine content.

Table 4.1. Mean and range of genetic distances between lines within barley landraces as revealed by SDS-PAGE of seed storage proteins

		Genetic distance		Frequency (%)				
No	Landraces	Range	Mean	<0.50	0.50-0.70	>0.70	Identical pairs (%)	Locality
1.	Acc.976	0.000-0.790	0.618	11	60	29	1	Wuchale
2.	Acc.1017	0.342-0.840	0.606	23	55	22	-	Kimbibit
3.	Acc.1153	0.420-0.874	0.634	13	61	26	-	Were Jarso
4.	Acc.1182	0.387-0.866	0.634	6	65	29	-	Wuchale
5.	Acc.144	0.242-0.845	0.622	15	62	23	-	Kimbibit
6.	Acc.1552	0.229-0.888	0.625	13	56	31	-	Girar Jarso
7.	Acc.1822	0.218-0.845	0.635	8	72	20	-	Girar Jarso
8.	Acc.2812	0.00-0.919	0.608	26	46	28	1	Wuchale
9.	Acc.296	0.346-0.894	0.664	9	53	38	-	Girar Jarso
10.	Acc.3151	0.408-0.866	0.618	12	56	32	-	Kuyu
1.1.	Acc.3395	0.408-0.841	0.663	5	53	42	-	Kimbibit
12.	Acc.3676	0.408-0.912	0.678	5	47	48	-	Kuyu
13.	Acc.3679	0.235-0.881	0.571	40	25	35	-	Girar Jarso
14.	Acc.4319	0.258-0.856	0.640	10	58	32	-	Kuyu
15.	Acc.4601	0.288-0.912	0.659	9	44	47	-	Kuyu
16.	Acc.4959	0.447-0.806	0.624	6	67	28	-	Girar Jarso
17.	Acc.4964	0.267-0.845	0.581	22	59	19	-	Girar Jarso
18.	Acc.4970	0.353-0.829	0.614	9	68	23	-	Girar Jarso
19.	Acc.4993	0.342-0.907	0.630	18	52	30	-	D. Libanose
20.	Acc.987	0.365-0.856	0.646	6	63	31	-	D. Libanose
21.	Baleme	0.000-0.935	0.648	13	35	52	4	Welmera
22.	Bukura	0.000-0.881	0.611	30	21	49	9	Kimbibit
23.	Demoye	0.000-0.816	0.356	62	22	16	34	Kimbibit
24.	Feleme	0.000-0.894	0.494	42	26	32	24	Kimbibit
25.	Feres Gama	0.000-0.100	0.474	59	11	30	24	Kimbibit
26.	Kessele	0.000-0.816	0.394	63	22	15	24	Kimbibit
27.	Mage	0.000-0.935	0.565	17	50	33	8	Kimbibit
28.	Nech Ferke	0.000-0.845	0.547	32	45	23	7	Ank-Mez
29.	Nech Gebs	0.000-0.866	0.565	28	7	65	28	Kimbibt
30.	Tikur Gebs	0.000-0.866	0.353	46	19	35	46	Ank-Mez
31.	Tolese	0.000-0.942	0.575	37	22	41	1	Degem
32.	Acc.1551	0.000-0.886	0.564	29	51	20	1	Girar Jarso
33.	Haddo	0.000-0.881	0.585	30	39	31	6	Degem
34.	Acc.653	0.235-0.881	0.642	8	64	28	-	Girar Jarso
35.	Acc.1156	0.229-0.888	0.641	18	52	30	-	Were Jarso
36.	Acc.984	0.267-0.755	0.551	31	57	12	-	D. Libanose
37.	Acc.973	0.000-0.894	0.530	45	32	23	2	Wuchale
38.	Acc.1609	0.000-0.894	0.373	63	31	6	34	Kimbibit
39.	Acc.1570	0.267-0.886	0.615	19	51	30	-	Girar Jarso
40.	Acc.1814	0.267-0.886	0.632	12	50	38	-	Girar Jarso
41.	Y. Gebs	0.000-1.000	0.561	43	19	38	16	Ank-Mez
42.	Key Ferke	0.000-1.000	0.574	39	27	34	11	Ank-Mez
43.	Acc.659	0.272-0.838	0.610	17	58	25	-	Girar Jarso
44.	Acc.4320	0.288-0.912	0.670	9	40	51	-	Kuyu

Ank-Mez=Ankober-Mezezo

4.4.2. *Variability among landraces*

Mobilities 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 (chapter III) and electrophoresis data. The mean genetic distance estimate among all landraces was 0.640, values ranging from 0.235 to 0.881. Only 12.24 % of the 1275 pair-wise comparisons have genetic distance values $\leq$ 0.500 and 29.33 % have 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 4.3) 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 and Welmera (0.405$\pm$0.05), Were Jarso and Kuyu (0.555$\pm$0.12) and Wuchale and Debre Libanose (0.568) (Table 4.4). On the other hand distant genetic relationships were observed between landraces of Girar Jarso and Were Jarso (0.704$\pm$0.07), and that between landraces from Wuchale, Kimbibit, Ankober-Mezezo, and Kuyu with that of landraces from Degem/Welmera. Thirty five (53 %) out of the 66 pair-wise comparisons with the highest genetic distance were comprised of landraces of Degem plus Welmera vs landraces from other localities mainly from Kimbibit and Ankober-Mezezo. Genetic distances among landraces within localities were generally lower than among localities (Table 4.4) and the lowest mean value (0.457$\pm$0.06) was observed among landraces at Debre Libanose. Only two landraces (Kessele vs acc.1609(6R) from Kimbibit exhibited very high genetic distance. Distantly and closely related landraces sorted out of the 1275 pair-wise genetic distance comparisons are presented in Table 4.2. 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 cannot be a reflection of desirable agronomic features. Hence, evaluation for important agronomic characters and crossing among those distantly related landraces will help combine traits of economic significance and broad segregation of the characters concerned.

The magnitude of genetic variability within some of the landraces 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 the within landraces component.

Table 4.2. Genetic distances among barley landraces (by spike types) from north Shewa, Ethiopia, as reavealed by SDS-PAGE of seed storage proteins

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
1.296	0.42	0.59	0.64	0.59	0.64	0.54	0.68	0.64	0.68	0.72	0.68	0.64	0.48	0.64	0.59	0.54	0.48	0.59	0.54	0.68	0.59	0.64	0.68	0.68	0.68	0.68	0.42	0.68	0.59	0.68	0.64	0.48	0.68	0.68	0.59	0.64	0.68	0.68	0.68	0.64	0.72	0.68	0.68	0.59	0.72	0.72	0.72	0.76	0.68	0.76
2.653		0.42	0.48	0.42	0.59	0.48	0.54	0.59	0.54	0.59	0.54	0.59	0.54	0.59	0.72	0.59	0.54	0.54	0.48	0.54	0.42	0.59	0.72	0.72	0.72	0.72	0.59	0.64	0.64	0.54	0.59	0.64	0.64	0.64	0.42	0.68	0.72	0.72	0.64	0.59	0.68	0.72	0.64	0.64	0.59	0.68	0.68	0.72	0.64	0.64
3.659			0.42	0.34	0.42	0.42	0.68	0.42	0.34	0.64	0.34	0.54	0.59	0.64	0.68	0.64	0.68	0.59	0.54	0.59	0.48	0.42	0.68	0.68	0.68	0.68	0.64	0.68	0.48	0.48	0.64	0.76	0.59	0.59	0.48	0.72	0.76	0.68	0.68	0.64	0.72	0.76	0.68	0.59	0.54	0.54	0.54	0.59	0.48	0.48
4.1551				0.23	0.47	0.52	0.66	0.52	0.57	0.47	0.57	0.52	0.57	0.62	0.66	0.7	0.66	0.52	0.62	0.52	0.66	0.62	0.81	0.74	0.74	0.74	0.78	0.81	0.66	0.66	0.7	0.74	0.57	0.57	0.47	0.7	0.66	0.66	0.66	0.62	0.62	0.66	0.57	0.57	0.52	0.62	0.66	0.62	0.62	0.52
5.1552					0.52	0.47	0.62	0.57	0.52	0.52	0.52	0.47	0.52	0.57	0.7	0.66	0.62	0.57	0.57	0.57	0.62	0.57	0.78	0.78	0.7	0.7	0.74	0.78	0.62	0.62	0.66	0.7	0.52	0.52	0.4	0.66	0.7	0.62	0.62	0.57	0.66	0.7	0.62	0.52	0.57	0.66	0.62	0.66	0.57	0.57
1,570						0.4	0.74	0.4	0.47	0.57	0.47	0.62	0.66	0.7	0.66	0.62	0.66	0.52	0.62	0.52	0.57	0.52	0.66	0.57	0.66	0.66	0.62	0.74	0.57	0.66	0.78	0.81	0.66	0.66	0.66	0.84	0.74	0.74	0.81	0.7	0.78	0.66	0.66	0.66	0.52	0.4	0.57	0.52	0.4	0.52
7.1814							0.62	0.47	0.4	0.62	0.4	0.47	0.52	0.57	0.7	0.57	0.52	0.66	0.57	0.66	0.52	0.47	0.62	0.62	0.62	0.62	0.57	0.7	0.52	0.62	0.74	0.7	0.52	0.52	0.52	0.74	0.7	0.62	0.7	0.57	0.74	0.62	0.62	0.52	0.57	0.47	0.62	0.66	0.47	0.66
8.1822								0.7	0.57	0.57	0.66	0.62	0.57	0.52	0.66	0.52	0.57	0.62	0.52	0.62	0.57	0.7	0.66	0.74	0.66	0.57	0.62	0.66	0.66	0.57	0.62	0.66	0.47	0.57	0.47	0.52	0.66	0.57	0.47	0.52	0.62	0.57	0.57	0.66	0.62	0.78	0.81	0.84	0.78	0.78
9.3679									0.4	0.62	0.4	0.57	0.52	0.66	0.62	0.57	0.62	0.57	0.57	0.57	0.52	0.47	0.7	0.62	0.7	0.7	0.57	0.62	0.4	0.62	0.74	0.78	0.62	0.62	0.62	0.74	0.62	0.62	0.7	0.57	0.66	0.62	0.52	0.52	0.47	0.47	0.7	0.66	0.57	0.57
10.3679(I)										0.66	0.33	0.52	0.57	0.62	0.66	0.52	0.66	0.62	0.52	0.62	0.47	0.4	0.57	0.57	0.66	0.57	0.52	0.66	0.33	0.57	0.62	0.74	0.47	0.57	0.57	0.7	0.74	0.57	0.66	0.62	0.7	0.74	0.66	0.57	0.52	0.52	0.57	0.62	0.52	0.52
11.4959											0.57	0.52	0.57	0.52	0.81	0.7	0.66	0.52	0.62	0.52	0.66	0.7	0.88	0.81	0.81	0.81	0.78	0.74	0.74	0.66	0.78	0.81	0.57	0.57	0.47	0.7	0.66	0.74	0.57	0.4	0.62	0.57	0.47	0.66	0.62	0.7	0.74	0.7	0.62	0.62
12.4970(I)												0.4	0.57	0.62	0.74	0.62	0.66	0.62	0.52	0.62	0.47	0.4	0.66	0.66	0.66	0.66	0.62	0.66	0.47	0.57	0.7	0.81	0.57	0.57	0.57	0.78	0.74	0.66	0.66	0.52	0.7	0.74	0.66	0.57	0.52	0.52	0.57	0.62	0.4	0.52
13.4970(2R)													0.4	0.47	0.7	0.66	0.62	0.57	0.47	0.66	0.62	0.47	0.7	0.7	0.62	0.62	0.66	0.78	0.52	0.7	0.66	0.7	0.4	0.4	0.52	0.66	0.62	0.52	0.62	0.47	0.66	0.7	0.62	0.4	0.57	0.66	0.7	0.74	0.57	0.66
14.973														0.4	0.66	0.52	0.47	0.52	0.4	0.62	0.57	0.52	0.74	0.74	0.66	0.66	0.52	0.66	0.47	0.66	0.62	0.57	0.47	0.47	0.47	0.52	0.47	0.47	0.57	0.4	0.62	0.57	0.47	0.33	0.62	0.7	0.81	0.84	0.7	0.78
15.4964															0.78	0.66	0.52	0.57	0.47	0.66	0.62	0.57	0.78	0.78	0.62	0.7	0.66	0.62	0.62	0.62	0.66	0.7	0.4	0.23	0.4	0.57	0.52	0.62	0.62	0.47	0.66	0.52	0.4	0.4	0.66	0.74	0.84	0.88	0.74	0.81
16.1182																0.52	0.66	0.62	0.62	0.7	0.74	0.7	0.57	0.57	0.57	0.47	0.52	0.81	0.57	0.74	0.62	0.57	0.66	0.74	0.74	0.62	0.66	0.57	0.66	0.78	0.62	0.66	0.74	0.66	0.62	0.7	0.74	0.7	0.78	0.7
17.976																	0.4	0.57	0.47	0.57	0.52	0.66	0.62	0.62	0.62	0.52	0.33	0.62	0.52	0.7	0.57	0.52	0.62	0.7	0.62	0.66	0.7	0.62	0.62	0.57	0.66	0.62	0.62	0.62	0.57	0.66	0.7	0.74	0.66	0.74
18.2812																		0.62	0.52	0.62	0.57	0.7	0.74	0.74	0.57	0.66	0.52	0.57	0.66	0.74	0.7	0.57	0.66	0.57	0.57	0.7	0.57	0.66	0.66	0.52	0.62	0.47	0.47	0.47	0.62	0.7	0.81	0.84	0.7	0.84
19.984																			0.33	0.33	0.52	0.57	0.7	0.62	0.62	0.62	0.57	0.7	0.62	0.7	0.57	0.7	0.62	0.62	0.62	0.74	0.62	0.7	0.78	0.66	0.74	0.7	0.62	0.62	0.47	0.66	0.78	0.74	0.66	0.66
20.987(IR)																				0.47	0.4	0.47	0.62	0.62	0.52	0.52	0.47	0.62	0.52	0.62	0.47	0.62	0.52	0.52	0.52	0.66	0.62	0.62	0.7	0.57	0.74	0.7	0.62	0.52	0.47	0.66	0.78	0.81	0.66	0.74
21.987(6R)																					0.4	0.57	0.7	0.62	0.62	0.7	0.66	0.7	0.7	0.62	0.57	0.7	0.7	0.7	0.62	0.74	0.62	0.7	0.78	0.66	0.74	0.7	0.62	0.7	0.47	0.57	0.7	0.66	0.66	0.66
22.4993																						0.4	0.57	0.57	0.57	0.66	0.52	0.57	0.57	0.47	0.52	0.66	0.66	0.66	0.57	0.7	0.66	0.66	0.74	0.62	0.78	0.74	0.66	0.66	0.52	0.52	0.66	0.7	0.62	0.7
23.1153																							0.52	0.52	0.52	0.62	0.57	0.7	0.4	0.52	0.57	0.7	0.52	0.52	0.62	0.66	0.62	0.52	0.78	0.66	0.81	0.78	0.7	0.52	0.57	0.47	0.62	0.66	0.57	0.66
24.1156(IR)																								0.33	0.47	0.33	0.52	0.74	0.57	0.66	0.52	0.57	0.66	0.74	0.81	0.7	0.74	0.57	0.81	0.84	0.84	0.81	0.88	0.74	0.62	0.62	0.57	0.62	0.62	0.7
25.1156(6R)																									0.57	0.47	0.52	0.74	0.57	0.74	0.52	0.57	0.66	0.74	0.81	0.78	0.74	0.66	0.88	0.84	0.84	0.81	0.81	0.74	0.52	0.52	0.57	0.52	0.62	0.62
26.3151(6R)																										0.47	0.62	0.74	0.66	0.66	0.62	0.66	0.66	0.57	0.74	0.7	0.57	0.57	0.81	0.78	0.78	0.66	0.74	0.57	0.62	0.62	0.74	0.78	0.7	0.84
27.3676																											0.52	0.74	0.57	0.74	0.52	0.57	0.57	0.66	0.74	0.7	0.74	0.57	0.74	0.78	0.78	0.74	0.81	0.66	0.52	0.7	0.66	0.7	0.62	0.7
28.4319																												0.62	0.4	0.7	0.57	0.52	0.62	0.7	0.7	0.66	0.7	0.62	0.7	0.66	0.74	0.7	0.7	0.62	0.66	0.66	0.7	0.74	0.66	0.74
29.432																													0.66	0.66	0.7	0.74	0.74	0.66	0.66	0.78	0.74	0.81	0.74	0.62	0.78	0.66	0.57	0.66	0.62	0.7	0.81	0.84	0.7	0.78
30.4601																														0.66	0.62	0.66	0.47	0.57	0.66	0.62	0.66	0.47	0.66	0.62	0.7	0.74	0.66	0.47	0.62	0.62	0.66	0.7	0.62	0.62
31.Mage																															0.62	0.74	0.66	0.66	0.47	0.52	0.66	0.66	0.57	0.62	0.7	0.66	0.66	0.74	0.7	0.62	0.66	0.7	0.7	0.7
32.Kessele																																0.4	0.62	0.7	0.62	0.57	0.7	0.62	0.7	0.74	0.74	0.84	0.78	0.7	0.57	0.74	0.62	0.66	0.74	0.66
33.T.Gebs																																	0.66	0.74	0.66	0.52	0.66	0.57	0.66	0.7	0.7	0.74	0.74	0.66	0.7	0.78	0.66	0.7	0.78	0.78
34.F.Gama																																		0.33	0.47	0.52	0.66	0.47	0.57	0.52	0.7	0.66	0.57	0.47	0.62	0.7	0.74	0.78	0.7	0.7
35.Bukura																																			0.47	0.62	0.57	0.57	0.66	0.52	0.7	0.57	0.47	0.33	0.62	0.7	0.81	0.84	0.7	0.78
36.Feleme																																				0.52	0.66	0.66	0.47	0.4	0.62	0.57	0.47	0.57	0.62	0.7	0.74	0.78	0.7	0.7
37.Demoyer																																					0.52	0.4	0.4	0.57	0.57	0.62	0.62	0.62	0.81	0.81	0.78	0.81	0.88	0.81
38.K.Ferke																																						0.47	0.66	0.62	0.52	0.47	0.47	0.47	0.7	0.7	0.88	0.84	0.84	0.84
39.3395																																							0.57	0.62	0.62	0.66	0.66	0.47	0.7	0.7	0.74	0.78	0.78	0.78
40.1017																																								0.4	0.4	0.57	0.57	0.66	0.78	0.84	0.74	0.78	0.78	0.7
41.144																																									0.57	0.52	0.4	0.52	0.66	0.74	0.78	0.81	0.66	0.74
42.1609(IR)																																										0.52	0.52	0.62	0.74	0.81	0.78	0.74	0.81	0.66
43.1609(6R)																																											0.33	0.57	0.7	0.7	0.88	0.84	0.78	0.84
44.N.Ferke(6R)																																												0.47	0.62	0.7	0.88	0.84	0.78	0.78
45.N.Ferke(IR)																																													0.62	0.7	0.81	0.84	0.7	0.78
46.Y.Gebs																																														0.57	0.7	0.66	0.57	0.57
47.Tolese																																															0.62	0.57	0.57	0.66
48.Baleme(2R)																																																0.23	0.4	0.4
49.Baleme(IR)																																																	0.47	0.33
50.Haddo(6R)																																																		0.47

Table 4.3. 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-MEZxWelmer
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 distance (GD)

Table 4.4. Comparison of genetic distances (mean ±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

4.4.3. Cluster analysis

Hierarchal cluster analysis on the basis of genetic distances produced nine main clusters each consisting two to 11 landraces and four landraces [acc.4320, Mage, and acc.1182 and Haddo(6R)] appeared in their own in separate clusters (Fig.4.2). 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. 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 cannot be a factor to bring about such differences in grouping.

4.4.4. Association of data from SDS-PAGE and morphology

Correlation analysis between estimates of genetic distance based on hordein data and morphology based distance values (chapter III) 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 hordein data and that of within landraces genetic variability based on the Shanon Weaver mean diversity index values (H'). Clusters based on hordein and morphology data 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 one from morphological data and cluster six from SDS-PAGE while Nech Ferke(6R), Nech Ferke(IR) and Demoye(IR) in cluster three and cluster one from morphological and SDS-PAGE data, respectively.

Similar studies in barley (Bekele, 1984; Asfaw, 1989b; Ruiz et al., 1997; Schut & Stam, 1997) and in durum wheat (Tsegaye et al., 1994; 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 closer genetic relationships may be assumed within six-rowed or two-rowed barley types 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 between two-rowed and six-rowed barley types 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 if a closer genetic relationship was observed between the naked and covered types than vise versa. As a consequence, the variation not evident by morphological characters was revealed by hordein polypeptide banding patterns. On the other hand, proteins usually sample only variation which is caused by genes on a few chromosomes compared to quantitative morphological characters which samples a range of chromosomes which are not necessarily the same as for proteins. 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 characters (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).

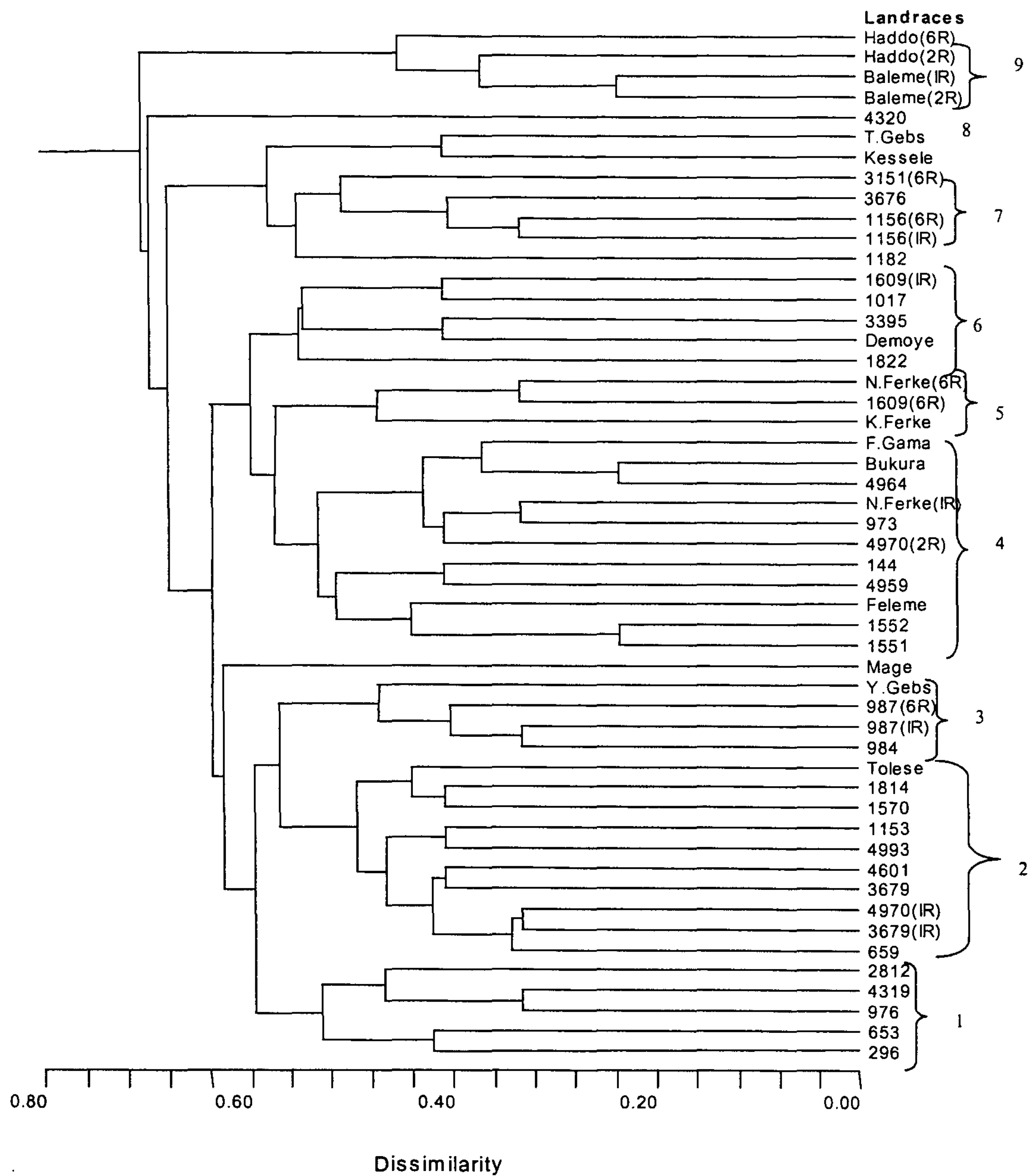

Figure 4.2. Dendrogram showing genetic relationships among landraces from north Shewa, Ethiopia following cluster analysis of data from SDS-PAGE of seed storage proteins

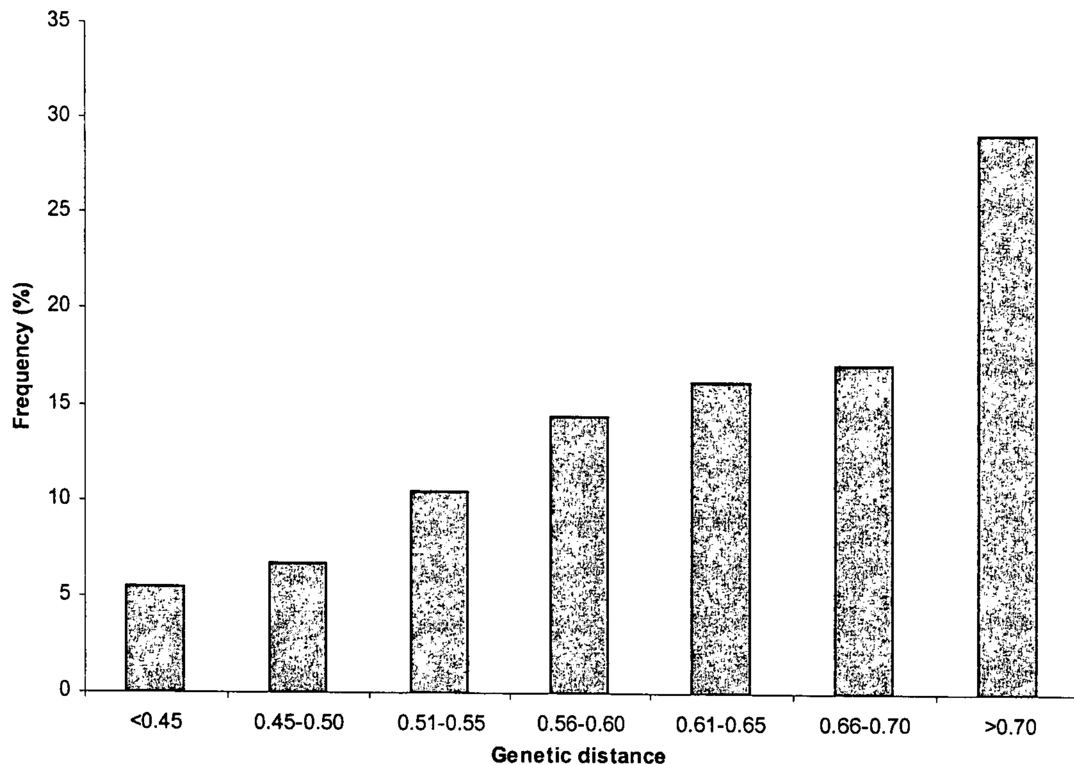

Figure 4.3. Frequency (%) distribution of genetic distances values among barley landraces from pair-wise comparisons resulting from SDS-PAGE of seed storage proteins.

4.5. 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 an 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 ± 0.11 for Were Jarso to 0.615 ± 0.10 for Ankober-

Mezezo) than mean genetic distance between landraces of different localities whose values range from 0.405±0.05 for landraces of Degem vs Welmera to 0.758±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 throughout 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. The first step in the utilization process would be to dissolve landraces that visually appear superior and at the same time are genetically heterogeneous, as revealed by SDS-PAGE, into their components descended from single spikes followed by performance evaluation under field conditions to isolate the best performing lines. The second step would be to identify parental lines with desirable agronomic traits from evaluation data and make crosses among those distantly related parents. Such approach will aid in predicting progeny performance and may estimate the degree of heterosis in the progeny of some parental combinations. Divergence for morphological traits *per se* may not reflect the true genetic distance between landraces. Hence, selection of parents for crossing shall 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.

CHAPTER V

AFLP ANALYSIS OF GENETIC RELATIONSHIPS BETWEEN BARLEY (*Hordeum vulgare* L.) LANDRACES FROM NORTH SHEWA IN ETHIOPIA.

5.1. Abstract

Local cultivars adapted to specific environmental conditions are the chief source of seed for farmers and therefore deserve research priority. The aim of this study was, therefore, to determine the genetic relationships between different barley landraces, predominantly farmers' cultivars, from north Shewa in Ethiopia so as to differentiate cultivars known by different local names and facilitate their conservation and use in breeding new varieties. Six AFLP primer combinations were analyzed across 19 barley landraces (14 farmers' cultivars and five accessions) and five malting varieties. The number of scoreable fragments amplified by each AFLP primer combination varied from 49 to 118 with an average of 84.5 and polymorphic fragments for each primer combination varied from 27 to 77 with an average of 58.5. The average percent polymorphism was 69.9 % with values ranging from 55.1 % to 75.8 %. Cluster analysis separated the 19 landraces and five malting varieties into two main clusters: the accessions and malting varieties into one cluster while all the farmers' cultivars, with the exception of two, were in the other main cluster. Mean genetic distance between all materials tested was 0.567, with values ranging from 0.317 to 0.745 while genetic distance between farmers' cultivars in particular ranged from 0.372 to 0.728 with an average of 0.545. Thirty one percent of the values in this range were $\leq$ 0.500 while 69 % of the values were $\geq$ 0.600 demonstrating the presence of sufficient variation between the farmers' cultivars. The lack of clustering of the accessions with the farmers' cultivars and the distinctness of cultivars from one another suggested that each cultivar is unique that may not be represented by the conventional germplasm sampling for conservation purposes. The close genetic relationship between the accessions and the four malting barley varieties and that of Stirling (malting variety) with Bukura (farmers' cultivar), a geographically isolated landrace with no documented common genetic background, could not be explained.

5.2. Introduction

Until recently, biochemical markers such as seed storage proteins and isozymes have been used to describe genetic variability and genetic relationships between germplasm and have contributed much to conventional breeding. However, compared to DNA based approaches, isozymes and seed storage proteins generate less markers and are not as informative (Gepts, 1990). Recent developments in DNA-based profiling methods offer new opportunities for genotype assessment (Ellis et al., 1997; Breyne et al., 1999). The advantage of DNA-based profiling over isozymes and seed storage protein markers is that these techniques can identify a large number of polymorphisms with good coverage of the entire genome. Analysis of DNA sequence polymorphisms have the potential to reveal sequence modifications such as silent nucleotide substitutions, insertions, or deletions in coding or flanking regions, or sequence divergence in non-expressed DNA such as repeated sequences, not previously possible to detect using conventional methods (Gepts, 1990).

Among the different DNA-profiling methods, polymerase chain reaction (PCR)-based marker techniques such as random amplified polymorphic DNA (Williams et al., 1990), simple sequence repeat polymorphism (Akkaya et al., 1992; Wu & Tanksley, 1993), and AFLP (Vos et al., 1995) provide new opportunities for assessing genetic diversity within and between different plant varieties and for characterizing and describing germplasm (Breyne et al., 1999). AFLP is a relatively new molecular technique for fingerprinting DNA of any origin and complexity and has several advantages over other DNA fingerprinting systems. Some of its advantages are possibility to detect small sequence variations using small quantities of genomic DNA (0.05 to 0.5μg), the ability to reveal many polymorphic loci per assay and the simultaneous analysis of numerous genotypes than other DNA-based techniques. The markers are reliable and reproducible between laboratories (Blears et al., 1998).

Amplified fragment length polymorphism (AFLP) has previously been used to assess genetic relationships in barley germplasm (Ellis et al., 1997; Russell et al., 1997; Schut et al., 1997) and was found to be useful in uniquely identifying different genotypes. The use

of DNA fingerprinting to assess genetic variability (Demissie, 1998) and genetic distinctness (Bjornstad, et al., 1997) in Ethiopian barley landraces sampled from different agro-ecological zones and altitudinal ranges were carried out. Local cultivars adapted to specific environmental conditions are the chief source of seed for farmers and deserve research priority. Therefore, the aim of this study was to determine the genetic relationships between different barley landraces, predominantly farmers' cultivars, from north Shewa in Ethiopia to clearly differentiate cultivars known by different local names and thereby facilitate their conservation and use in a breeding program.

5.3. Materials and methods

5.3.1. DNA extraction

A total of 19 landraces (14 farmers' cultivars and five accessions which were partly in common to those included in the previous studies) were used for this study (Table 5.1). Five South African malting barley varieties were also included for assessment. Five plants of each landrace were grown under greenhouse conditions at the University of Free State, Bloemfontein, South Africa. Extraction of DNA was carried out from five individual plants of each landrace using the method of Edwards et al. (1991) with some modification. Fresh and healthy leaf material of each plant was frozen in liquid nitrogen. and ground to powder with a mortar and pestle followed by the addition of 10 ml extraction buffer (0.01M NaCL, 0.05M Tris-HCL (pH 8.0), 0.01M EDTA (ethylenediamine tetra acetic acid (pH 8.0), and 1 % (w/v) SDS) pre-heated to 65°C. The samples were incubated at 65°C for 30 minutes with shaking every 10 minutes. CTAB (1 ml) (cetyltrimethylammonium bromide) and 2 ml of 5M NaCl was added for every 10 ml of extraction buffer and the homogenate was incubated for a further hour. Chloroform extraction were performed twice by the addition of isoamylalcohol (24:1) and centrifuged at 10 000 rpm for 15 minutes at 20°C. The supernatant was retained and the DNA precipitated by the addition of two volumes of ethanol and stored overnight at 4°C. The precipitated DNA was washed three times with 70 % ethanol. The washed DNA was redissolved in sterile distilled water and stored at 4°C. The concentration of DNA was measured with an UV spectrophotometer at 260 and purity

at 280 nm and as well as gel electrophoresis. Finally, DNA from individual plants representing each landrace was bulked to give a final working sample of 100 ng DNA/μl.

Table 5.1. List of farmers' cultivars and accessions included in the AFLP study

No	Cultivar/ Landrace	Origin	Description	No.	Cultivar/Landrace	Origin	Description
1	Mage	Kimbibit	Irregular spike, dull white seeds, early maturity	13	Haddo	Degem	Consists all categories of spike types, white seed color
2	Feres Gama	Kimbibit	Six-rowed, white seeds, late maturity	14	Yeferenge Gebs	Ankober-Mezezo	Six-rowed type, many similarities with Feres Gama and Nech Gebs
3	Nech Gebs	Kimbibit	Six-rowed, white seeds, late maturity	15	Acc. 1017	Kimbibit	Both six-rowed and irregular t spikes with gray seed color
4	Tikur Gebs	Ankober-Mezezo	Six-rowed, black seeds, early maturity	16	Acc.3395	Kimbibit	Six-rowed type with white seed color
5	Kessele	Kimbibit	Six-rowed, black seeds, early maturity	17	Acc. 144	Kimbibit	Six-rowed type with white seed color
6	Bukura	Kimbibit	Six-rowed, white seed with short spike, awns partially brittle when matured	18	Acc. 1609	Kimbibit	Comprised both six-rowed and irregular spikes with white seed color
7	Feleme	Kimbibit	Six-rowed, white seeds	19	3391-15	Kimbibit	A six-rowed pure line with black seed
8	Demoye	Kimbibit	Predominantly six-rowed with rare irregular spikes, purple type of seeds	20	Clipper	South Africa	Two-rowed, white seed color, very early maturing compared to the landraces
9	Key Ferke	Ankober-Mezezo	Predominantly six-rowed type with red veination on immature kernels, irregular types are also found	21	B94/2	South Africa	Two-rowed, white seed color, very early maturing compared to the landraces
10	Nech Ferke	Ankober-Mezezo	Similar morphological characters to Key Ferke but has white seeds	22	SSG 532	South Africa	Two-rowed, white seed color, very early maturing compared to the landraces
11	Tolese	Degem	Both two-rowed and irregular spikes, white seeds, late maturity	23	Schooner	South Africa	Two-rowed, white seed color, very early maturing compared to the landraces
12	Baleme	Welmera	Predominantly irregular spikes, dull white seeds	24	Stirling	South Africa	Two-rowed, white seed color, very early maturing compared to the landraces

5.3.2. AFLP reaction

Digestion and ligation

Restriction digestion was performed in 2 µl of EcoR I and Mse I at 37°C for two hours. Following the digestion, the restriction endonucleases were inactivated at 70°C for 15 minutes. The restricted fragments were then ligated with EcoR I and Mse I adapters using 1 µl T4 DNA ligase for two hours at 20±2°C. Following ligation, the reaction mixture was diluted 10 times in TE buffer and mixed very well to be used in subsequent PCR amplification.

PCR amplification

Two consecutive PCR amplification were performed (pre-selective and selective). In the pre-selective amplification, the 10x diluted ligation mixture was amplified with two AFLP primers each with one added selective nucleotide. In this step, 5 µl of diluted template DNA, 40 µl pre-amp primer mix, 5 µl 10x PCR buffer for AFLP containing MgCl and 0.2 µl Taq and 0.8 µl Sabax were used to make up 51 µl. The reaction mixture was mixed gently and centrifuged briefly. Pre-amplification was carried out for 20 cycles each cycle having a 30 second DNA denaturation step at 94°C, a one minute annealing step at 56°C, and a two minutes extension step at 72°C. Following, 12 µl of the reaction mixture was run on 1 % agarose gel (0.5g agarose, 50ml 0.5x TAE, and 0.5 µl ethidium bromide) at 80 mV to confirm pre-amplification.

The pre-selective amplification product was diluted 50 times. Selective AFLP amplification was carried out with six primer combinations (Eco+ACC/MSe+ CTC; Eco+ACC/Mse+CTA; Eco+AAC/Mse+CAA; Eco+ACT/Mse+CTC; Eco+ACT/ Mse+CTA; and Eco+ACA/Mse+CAA) each containing three selective nucleotides. The EcoR I primers were fluorescently labeled as NED (black) and FAM (blue). Components for PCR amplification were 5 µl of 50 times diluted template DNA (i.e, from 1:50 dilution of the pre-selective amplification product), 5.5 µl of Mix1 (4.5 µl Mse I primer and 1 µl EcoR I), 9.5 µl of Mix 2 (7.4 µl AFLP grade water, 2 µl 10x PCR buffer and 0.1 µl Taq DNA polymerase) which make up a total of 20 µl. The Mse I primer contained dNTPs. This reaction mixture was centrifuged briefly and

put in the PCR machine (Gen amp PCR system 2700 Applied Biosystems, hot lid thermocycler) to perform a total of 23 cycles of amplification. The first cycle was carried out at 94°C for 30 seconds, at 65°C for 30 seconds and at 72°C for two minutes. The annealing temperature was lowered by 0.7°C during the 12 cycles and then 23 cycles were carried out at 94°C for 30 seconds, at 56°C for 30 seconds and at 72°C for two minutes. After completion of selective amplification, 5 µl of the PCR product was added to 24 µl formamide (deionized) and 1 µl Rox (standard size marker) which is then denatured at 94°C for five minutes. The denatured DNA was rapidly cooled rapidly slowly in ice. Finally the DNA fragments were resolved with a Perkin Elmer Prism 310 automated capillary sequencer (PE Biosystem).

5.3.3. Data analysis

All the landraces and South African malting barley varieties were scored for presence or absence of polymorphic AFLP fragments, and the data were entered into a binary matrix as discrete variables ("1" for presence and "0" for absence of a similar fragment). Fragments larger than 50 base pairs were selected. Data were analyzed using NCSS 2000 program (Hintze, 1998). A dendrogram was created by cluster analysis by a weighted pair group method on the basis of arithmetic averages (UPGMA). A calculated cophenetic value matrix was compared with the actual data's matrix to evaluate the degree of fitness between the two matrices. The matrices generated from the six individual primer pairs and a matrix from a combination of the six primer pairs were compared to test for goodness of fit between two matrices. The relatedness between the two matrices was measured by Pearson correlation coefficient (r). Higher value of "r" indicates a higher degree of similarity.

5.4. Results and discussion

The number of scoreable fragments amplified by each AFLP primer combination varied from 49 for MseI+CTC/EcoRI+ACC to 118 by MseI+CAA/EcoRI+AAC with an average of 84.5 per primer combination (Table 5.2). A total of 351 polymorphic AFLP fragments were detected across the different landraces/varieties, with the number of polymorphic fragments for each primer pair varying from 27 (55.1 %) for MseI+CTC/EcoRI+ACC to 77

(76.2 %) for MseI+CTA/EcoRI+ACT with an average of 58.5 fragments per primer combination (69.9 %) (Table 5.2).

The average percent polymorphism obtained in this study using AFLPs is similar to the 62 % reported by Demissie et al. (1998) using RFLPs on barley landraces from Ethiopia. However, this is higher than the 48.8 percent polymorphism reported by Russell et al. (1997) using AFLPs, who also reported a corresponding percent polymorphism of 66.3 % using RAPDs, 100 % for SSRs and 83.2 % for RFLPs. The different levels of polymorphism detected in the different studies between different genotypes are a result of differences in the genotypes studied as well as the choice of primers for RAPDs, AFLPs and SSRs and restriction enzymes and probes for RFLPs.

It is interesting to note that the number of detected fragments for each landrace or variety differed considerably for each primer combination. For example, Mse I + CAA/EcoRI + ACA repeatedly detected only one single fragment in Tikur Gebs and Feres Gama, while all the other primers used detected on average as many fragments or higher as in the other accessions or cultivars (Table 5.3). Menkir et al. (1997) and Ruas et al. (1999) found similar results in assessing the genetic diversity of *Chenopodium* L and sorghum, respectively using RAPDs. This suggests that certain sequences may have very low genome coverage in specific genotypes.

Table 5.2. Total number of DNA fragments, total polymorphic fragments and percent polymorphic fragments resulting from six primer combinations (PC) in AFLP analysis of barley landraces from north Shewa in Ethiopia.

Primer combination	Number of fragments	Polymorphic fragments	Percent polymorphism
MseI+CTC/EcoRI+ACC	49	27	55.1
MseI+CTA/EcoRI+ACC	87	66	75.8
MseI+CAA/EcoRI+AAC	118	67	56.8
MseI+ CTC/EcoRI+ACT	80	51	63.7
MseI+CTA/EcoR+ACT	101	77	76.2
MseI+CAA/EcoR+ACA	72	55	76.4
Total	507	351	419.8
Average	84.5	58.5	69.9

Table 5.3. Number of DNA fragments produced by each of the six primer combinations from AFLP analysis of genetic relationships in barley landraces from north Shewa in Ethiopia.

No	Landraces/varieties	PC1	PC 2	PC 3	PC4	PC 5	PC 6	total	Mean
1	Mage	30	38	68	46	52	22	256	42.7
2	Kessele	29	44	65	39	60	39	276	46.0
3	Tikur Gebs	35	45	70	45	59	1	255	42.5
4	Feres Gama	36	46	56	51	60	1	250	41.7
5	Bukura	26	39	57	39	33	17	211	35.2
6	Feleme	32	35	57	49	49	23	245	40.8
7	Nech Gebs	34	40	70	56	50	16	266	44.3
8	Demoye	31	44	70	48	50	26	269	44.8
9	Key Ferke	29	32	71	48	46	28	254	42.3
10	Nech Ferke	30	32	71	41	47	40	261	43.5
11	Yeferenge Gebs	31	29	66	47	40	36	249	41.5
12	Tolese	30	40	70	44	44	35	263	43.8
13	Baleme	28	35	73	22	39	41	238	39.7
14	Haddo	29	38	58	45	41	26	237	39.5
15	Acc. 3395	28	35	68	46	44	38	259	43.2
16	Acc. 1017	28	35	62	41	36	31	233	38.8
17	Acc. 144	26	35	64	44	34	33	236	39.3
18	Acc. 1609	28	37	68	45	37	40	255	42.5
19	3395-15	29	35	63	40	31	40	238	39.7
20	Clipper	24	33	65	36	40	42	240	40.0
21	B 94/2	27	30	67	43	39	41	247	41.2
22	SSG 532	22	30	59	43	27	41	222	37.0
23	Schooner	21	29	58	46	39	43	236	39.3
24	Stirling	21	28	58	49	39	40	235	39.2
Mean		28.5	36.0	64.7	43.8	43.1	30.8	247.1	41.2

Note : PC 1 to PC 6 refer to the primer combinations in Table 5.2 listed in order.

Mean genetic distance between all materials tested was 0.567, values ranging from 0.317 between acc. 1609 and 3391-15 to 0.745 between Feres Gama and Clipper (Table 5.4). Mean genetic distance between farmers' cultivars in particular ranged from 0.372 to 0.728 with a mean of 0.545. Thirty one percent of the values in this range were ≤ 0.500 while 69 % of the pair wise comparisons had genetic distance values ≥ 0.600 demonstrating the presence of sufficient variation between the farmers' cultivars. Among the farmers' cultivars, Demoye vs Nech Gebs and Nech Ferke vs Key Ferke were the closest genetically while Feres Gama and Nech Ferke were distantly related (Table 5.4).

Cluster analysis using the UPGMA method of NCSS 2000 program (Hintze, 1998) separated the 19 landraces and the five malting varieties into two main clusters (A and B) with a high cophenetic correlation (0.868) between the actual distance and the predicted distance based on this method of hierarchical configuration. Group A comprised all the Kimbibit germplasm accessions, the South African malting varieties as well as three farmers' cultivars, Bukura, Key Ferke and Nech Ferke (Figure 5.1). Group B was made up of landraces from a variety of localities including Degem, Kimbibit, Welmera and Ankober-Mezezo. At 0.45 cluster cut-off, each of the broader groups was further branched to form sub groups. In group A, all accessions from Kimbibit and four of the five South African malting varieties formed separate sub groups. The two farmers' cultivars (Key Ferke and Nech Ferke) from Ankober-Mezezo area, in particular from Mush locality, also showed closer genetic affinity and appeared separately from the other sub groups within the main cluster. These two landraces showed closer heading and maturity days and comparable plant height based on data from field evaluation which will be discussed in chapter VI. Red veins or strips on the immature kernels of Key Ferke is a characteristic morphological feature that distinguishes it from Nech Ferke. It is interesting to see that all accessions from the same locality appeared in the same sub group; however, the closer genetic relationship between the accessions and the four malting barley varieties (B 94/2, SSG 532, Schooner and Clipper) and that of Stirling with Bukura that are geographically isolated and have no common genetic background could not be explained in view of the expectation that these accessions would have shown closer affinity to the farmers' cultivars in Group B where they co-existed and adapted to similar environmental conditions. On the other hand the clustering of all accessions from a similar locality into one sub group indicated that the accessions might have derived from similar or closely related populations. Hence, sampling germplasm in a given locality may not represent the whole array of genetic diversity of the locally grown farmers' cultivars. Although Bukura is in the same group with the South African malting varieties and the accessions, it has a greater degree of dissimilarity compared to the accessions within the same main cluster.

The landraces in group B consisted solely of farmers' cultivars from different geographic locations (Figure 5.1). Although the sub groupings seem more or less dependent on the

localities they came from, it was not consistent throughout all the sub groups. For example, Tikur Gebs from Ankober-Mezezo appeared with the two cultivars from Kimbibit; Yeferenge Gebs from Ankober-Mezezo area appeared with Baleme and Tolese from north-west Shewa; and Feres Gama from Kimbibit with Haddo that came from Degem. Moreover, morphological similarity between the cultivars did not seem to play a large role in the formation of the sub groups except for Kessele and Tikur Gebs, both early maturing with black seeds. It is assumed that these two cultivars are genetically the same but have been given different names by different ethnic groups in Ethiopia. Both names correspond to seed color, Kessele meaning as black as 'coal' in Oromic and Tikur Gebs 'black barley' in Amharic. Tikur Gebs is popular to Amhara nationality at Ankober-Mezezo area while Kessele is very well known by Oromo nationality at Kimbibit. Although not common, farmers in the Ankober-Mezezo area call Tikur Gebs as 'Ye Oromo Gebs' meaning barley of Oromo people to denote that this barley type was brought to their locality by Oromo people or it is commonly grown by Oromo people. This study has demonstrated that although these two cultivars are genetically related, they are not the same with a genetic distance of 0.415.

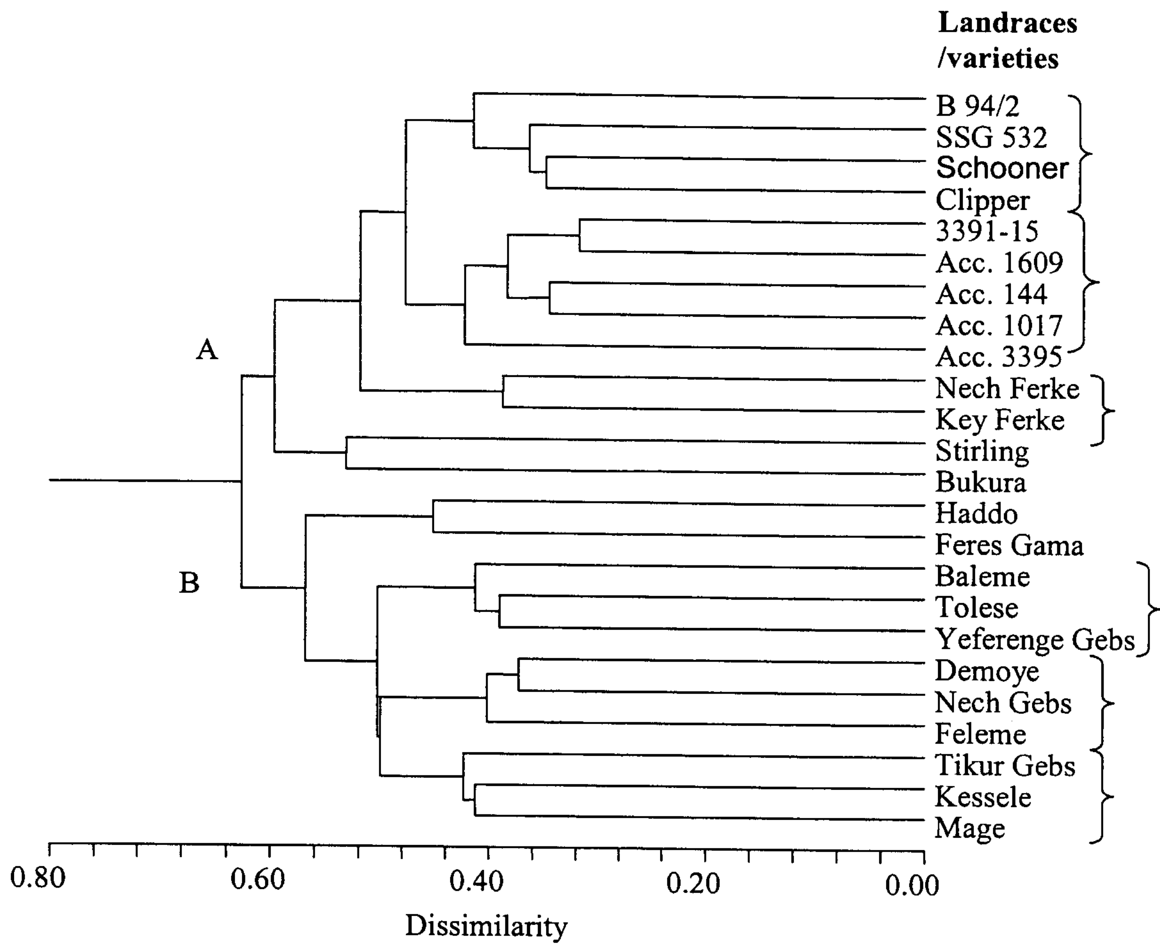

Figure 5.1. Dendrogram illustrating the genetic relationships between farmers' cultivars and accessions from north Shewa in Ethiopia as revealed by AFLP using six primer combinations.

Table 5.4. Actual genetic distance values between landraces from north Shewa in Ethiopia as revealed by AFLP using six primer combinations.

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	0.412	0.431	0.539	0.622	0.475	0.485	0.485	0.591	0.619	0.480	0.494	0.505	0.520	0.622	0.577	0.595	0.600	0.591	0.642	0.647	0.624	0.627	0.694
2		0.415	0.564	0.687	0.525	0.529	0.498	0.598	0.589	0.475	0.475	0.473	0.545	0.611	0.581	0.595	0.570	0.568	0.624	0.664	0.627	0.613	0.719
3			0.466	0.666	0.518	0.501	0.482	0.626	0.645	0.539	0.514	0.566	0.568	0.645	0.631	0.647	0.635	0.647	0.690	0.685	0.681	0.674	0.720
4				0.577	0.573	0.573	0.585	0.692	0.728	0.615	0.611	0.972	0.451	0.725	0.694	0.698	0.713	0.708	0.745	0.737	0.736	0.730	0.647
5					0.662	0.669	0.652	0.573	0.598	0.649	0.655	0.674	0.531	0.598	0.579	0.615	0.598	0.604	0.643	0.631	0.629	0.633	0.631
6						0.396	0.407	0.558	0.602	0.482	0.496	0.550	0.572	0.635	0.617	0.615	0.624	0.615	0.667	0.655	0.640	0.640	0.702
7							0.372	0.558	0.613	0.478	0.492	0.562	0.591	0.624	0.624	0.622	0.638	0.636	0.981	0.659	0.640	0.660	0.711
8								0.520	0.575	0.473	0.454	0.537	0.579	0.631	0.613	0.647	0.613	0.611	0.660	0.659	0.633	0.643	0.724
9									0.387	0.537	0.562	0.598	0.629	0.489	0.475	0.535	0.503	0.505	0.556	0.566	0.535	0.548	0.640
10										0.522	0.527	0.566	0.655	0.487	0.463	0.520	0.482	0.498	0.533	0.568	0.525	0.541	0.645
11											0.390	0.420	0.531	0.548	0.522	0.537	0.543	0.541	0.596	0.624	0.554	0.593	0.685
12												0.404	0.552	0.613	0.575	0.600	0.575	0.581	0.626	0.645	0.600	0.604	0.708
13													0.566	0.581	0.554	0.556	0.529	0.531	0.564	0.608	0.579	0.595	0.700
14														0.617	0.579	0.600	0.617	0.619	0.654	0.669	0.636	0.633	0.560
15															0.412	0.410	0.396	0.471	0.494	0.548	0.507	0.498	0.617
16																0.344	0.372	0.420	0.485	0.522	0.485	0.480	0.595
17																	0.344	0.396	0.463	0.516	0.482	0.478	0.585
18																		0.317	0.410	0.509	0.466	0.451	0.613
19																			0.390	0.489	0.423	0.439	0.604
20																				0.387	0.366	0.348	0.570
21																					0.420	0.436	0.482
22																						0.360	0.550
23																							0.512
24																							–

Note : Numbers 1 to 24 refer to the landraces and malting varieties included in the AFLP study as listed in Table 5.3.

Correlation analysis was performed between genetic distance values resulting from each primer combination in order to identify the primer combinations that contributed most to the overall genetic relationships between landraces (Figure 5.1). The analysis revealed that although data from each of the six primer combinations were significantly correlated with the combined data of the six primer combinations, the ability of each primer combination to distinguish the different landraces was significant. As a result, the dendrogram created based on just two primer combinations was similar to one using the combined data with only slight differences in the internal structure of sub groups (Figure not indicated). Although the important role of specific primer combinations for AFLP analysis has been indicated by Ellis et al. (1997), these data support the hypothesis of Russell et al. (1997) that the number of markers generated per AFLP primer combination does not necessarily correlate to percentage polymorphism identified.

Bjornstad et al. (1997) and Demissie et al. (1998) were the first to report on the application of DNA-fingerprinting (RFLPs) to assess variability in Ethiopian barley landraces collected from various parts of the country. This current study is the first application of DNA-fingerprinting using AFLPs in the quantification of genetic diversity between Ethiopian barley landraces comprising predominantly farmers' cultivars. This study has indicated that none of the farmers' cultivars were identical. Hence, the farmers' cultivars may contain rare alleles that are not represented by accessions in gene banks. Marshall (1989) also emphasized the importance of on-farm populations, in this case farmers' cultivars, to contain a much greater number of rare alleles and different (multilocus) genotypes than accessions in gene banks. This also suggests the need to conserve farmers' cultivars that may not be represented by conventional germplasm exploration.

Table 5.5. Correlation values between genetic distance matrices from different primers combinations revealed by AFLP analysis of genetic relationships in barley landraces from north Shewa in Ethiopia.

	PC2	PC3	PC4	PC5	PC6	All
PC1	0.231***	0.114NS	0.326***	0.233***	0.299***	0.391***
PC2		0.148**	0.232***	0.874***	0.259***	0.762***
PC3			0.196***	0.132*	0.364***	0.641***
PC4				0.204***	0.206***	0.406***
PC5					0.285***	0.764***
PC6						0.626***

PC=primer combination (as described for Table 5.2)

*P=0.05, **P=0.01, and ***P=0.001

5.5. Conclusions

Cluster analysis based on AFLP data from six primer combinations clearly indicated that none of the farmers' cultivars were genetically identical. The fact that the accessions were not clustered together with the farmers' cultivars implicated that each cultivar may possess adaptive gene complexes unique to it that may not be represented by random sampling of germplasm from areas of its production. The information from this study will be helpful for the current *in situ* biodiversity conservation strategy undertaken by the Biodiversity Conservation and Research Institute in Ethiopia in order to prevent the exclusion of cultivars that are morphologically similar but genetically distinct. Furthermore, a comprehensive study of all the farmers' barley cultivars, grown in different parts of Ethiopia, is required to maximize the efforts of germplasm conservation and utilization in national and regional breeding programs.

CHAPTER VI

GENETIC VARIABILITY AND YIELD RELATIONSHIPS OF BARLEY CULTIVARS FROM NORTH SHEWA IN ETHIOPIA

6.1. Abstract

Sixty two pure lines derived from landraces, mainly farmers' cultivars, were evaluated at two locations in north Shewa, Ethiopia during 2001. Urea and Diamonium Phosphate (DAP) fertilizers were applied at 41 and 46 kg ha^{-1} of N and P_2O_5, respectively at time of planting. Data for the agronomic characters were subjected to analyses of variance and cluster analysis to determine differences for the characters within and among landraces. Estimates of variance components, and genotypic and phenotypic correlations were computed following variance and covariance analyses. Interrelationships between quantitative characters were investigated based on path coefficient analysis to set criteria for selection. SDS-PAGE of seed storage proteins was carried out to complement morphological data. Estimates of genetic variances for the different characters indicated that number of seeds per spike, spike length, and grain yield per spike combined higher values of genotypic coefficients of variation (GCV) and broad sense heritability. Hence, these characters exhibited higher values of genetic advance (GA) ranging between 21.7 % for number of seeds per spike to 30 % for spike length and grain yield per spike. Selections within cultivars were tightly clustered together, in most cases, denoting less variation within than between farmers' cultivars. Accordingly, GA through selection (assuming 5 % selection intensity) for the different agronomic characters within clusters was very low. This was further supported by SDS -PAGE that revealed very low variability within farmers' cultivars compared to between cultivars. Data from SDS-PAGE and quantitative characters showed similar patterns of clustering and genetic distance values resulting from the two measures of variability were significantly correlated (r=0.199). Grain yield was strongly and positively associated with plant height, spike length, number of heads per unit area and harvest index. Path coefficient analysis revealed that high number of heads per square meter resulted in a low number of seeds per spike and grain yield per spike but it had the highest (0.792) positive direct effect on grain yield followed by number of seeds per spike, grain yield per spike and duration of grain filling period.

6.2. Introduction

Landraces are largely the outcome of natural and human selection during centuries of cultivation and usually exhibit genetic variation for qualitative and quantitative characters, have good adaptation to specific environmental conditions and give dependable yield (Harlan, 1992). The long history and extensive use of cultivation of barley in Ethiopia has given rise to a great number of farmers' cultivars that are adapted to specific environments. In self-pollinated crops such as barley, landraces could be utilised in two broad ways: first by developing cultivars through selection of homogeneous superior lines; secondly, the superior pure lines can be used in the crossing program as recipients of useful genes which may not be present in these adapted populations (Lakew et al., 1997). These approaches are vital to Ethiopian conditions where cultivation of landraces by subsistent farmers is still predominant because high demand for inputs and lack of adaptation to prevailing environmental stresses precluded the use of improved high yielding varieties by farmers.

Effective utilization of landraces requires their proper and systematic evaluation. Even though there has been success in the release of new varieties isolated from landraces, progress in selection for specific adaptation has been slower than anticipated in spite of the availability of germplasm adapted to different environments. It is possible that this has resulted partly from a gradual development of breeding principles and germplasm evaluation approaches for particular environments. The fact that quantitative agronomic characters are controlled by several genes makes the situation more complicated and the possibility of finding the best genotypes at all loci extremely small (Wricke & Weber, 1986). This is true because while selecting for quantitative agronomic characters, only phenotypes can be observed and the best genotype may be overlooked even if it is in the landrace.

Despite difficulties in selection for quantitative characters, phenotypic selection is one of the main processes in any breeding program largely dependent on empirical methods. Knowledge of the advance to be expected by applying selection pressure is useful in designing an effective breeding program. Selection depends on variation, and statistical parameters like means and variances are used to measure the response

to selection. This response depends not only on the genetic variance, but also on the non-genetic variance and interaction of the genetic effects with the environment (Wricke and Weber, 1986). The basic idea in studying variation is its partitioning into different causes. Moreover, characters like yield are much influenced by environmental effects and their heritability is therefore low. Hence, knowledge of interrelationships among different characters and their subsequent use in indirect selection for characters that are not easily measured or that exhibit low heritability is also important. Indirect selection can also be advantageous if the indirect character can be measured with more accuracy than the primary character and have higher heritability. Such research in Ethiopian barley landraces is lacking or scarce except that of Sinebo (2002) and Alemu (2001) and yet these studies are not concerned with farmers' cultivars. Variability studies have been more academic and few cases are known where the variability has been used in breeding programs in the areas where landraces are adapted (Lakew et al., 1997). The objectives of this study were: i) to study the variability for economically important characters present in farmers' cultivars from north Shewa in Ethiopia, ii) to determine their genetic relationships and iii) to evaluate their agronomic characters and see associations between characters that could be incorporated into selection programs in the future. The fact that the field evaluation was supported with data from SDS-PAGE of seed storage proteins makes the information reliable.

6.3. Materials and methods

6.3.1. Plant materials

In this study emphasis was given to predominantly farmers' cultivars grown in north Shewa. Two accessions collected by the Biodiversity Conservation and Research Institute, Ethiopia were also included. Ten random plants from each landrace were selected and seed increase was done during the off-season (February to May 2001 in Ethiopia) to get pure lines with sufficient seeds for the field experiment. The random selections within each landrace appeared very similar and for ease of management five lines from each landrace, except for Baleme and Key Gebs represented by one, and six lines, respectively were included in the experiment. Misrach (released

regionally for north Shewa) and HB-42 (released nationally) were included as checks for comparison. Total entries for evaluation were 62 lines and two checks.

6.3.2. Experimental procedure

The 62 lines and the two checks were planted at two locations, Keyit and Sheno in north Shewa, Ethiopia in the last week of June, 2001. Each entry was planted on a plot size of two rows of 2.5m length spaced 20cm between rows. A randomized complete block design with four replications was used at each location. Seed rate was 85kg ha^{-1}. Each plot received urea and Diamonium Phosphate (DAP) fertilizers at rates of 41 and 46kg ha^{-1} of N and P_2O_5, respectively at the time of planting. Weeds were controlled manually before they became critical for nutrient competition.

6.3.3. Data collection

Days to heading and maturity, biomass, and grain yield were recorded on a plot basis. A plot was judged to be at heading when heads were fully visible in 50 % of the plants in a plot. Physiological maturity was recorded when the peduncle turned yellow and plants showed complete leaf senescence in 90 % of the plants in a plot. Plant height, spike length, number of seeds per spike, and grain yield per spike were recorded from five randomly selected spikes in each plot and their means were used for statistical analysis. Number of productive heads were counted from a one-meter section of each plot and converted to spikes per square meter. Harvested plots were air-dried before measuring biomass and 25 grams of grain from each plot was oven dried at 130 ^{0}C for 2½ hours and grain yield was adjusted to 12.5 % moisture level to avoid possible differences in moisture content of grain harvested from each plot. Harvest index of each entry was obtained from the ratio of adjusted grain yield per plot to biomass per plot. Two hundred and fifty seeds were counted from each plot, weighed and converted to calculate 1000 seed weight. At Sheno, all the materials were planted on a camber bed to avoid waterlogging and all the data from this site were recorded from a 1.5m section of each plot by trimming one metre from the end to avoid irregularity in performance of plants within a plot.

6.3.4. Quantitative and seed storage protein variability

Simple statistics were used to indicate the mean and range of values along with the level of statistical significance for quantitative characters. The structure of phenotypic diversity was studied both within a cultivar and across cultivars bearing different names. To classify the farmers' cultivars, principal component analysis (PCA) and cluster analysis were performed. The quantitative characters were first scaled so that their variances are equal and principal component analysis was done on the standardized variables prior to the cluster analysis to distinguish the most important variables. Hierarchical cluster analysis was carried out using UPGMA (Unweighted Pair Group Method Using Arithmetic Means) based on Sneath and Sokal (1973). Moreover, for each numerical character, the distance between two lines was calculated as the difference in character values divided by the overall range for that character which puts the distance on a zero to one scale as described by Johns et al. (1997). To form a morphological distance matrix, the individual character distances for each pair of lines were summed and divided by the number of characters scored in both lines. Genetic relationships were further investigated using SDS-PAGE of seed storage proteins following the procedure described in chapter IV with protein extraction being done from two seeds of each pure line. All computations were done using Number Cruncher Statistical System (NCSS 2000) computer program.

6.3.5. Estimation of genetic variances

Analyses of variance (ANOVA) for each location and for data combined from the two locations were done using the MSTAT-C computer program. At Sheno the third replication was not good and analyses were done based on the data from the remaining three replications. Genotypic ($\delta^2 g$) and phenotypic (δ^2_P) variances for each character were estimated from the mean squares in the ANOVA according to Singh and Chaudhary (1985) and Wricke and Weber (1986) for individual locations and for the combined data of the two locations as $\delta^2_g = (MS_g - MSe)/r$ where MS_g and MSe are mean squares of genotypes and error, respectively and r is the number of replications. δ^2_g represents the component due to genetic differences among lines from landraces. The phenotypic variance was obtained taking the genotypic and error

variances into account as $\delta^2_p = \delta^2_g + \delta^2 e / r$ where $\delta^2 e$ is variance of error which includes plot effects, error due to sampling within plots, and errors of measurement.

Genotypic coefficient of variation *(GCV)* and phenotypic coefficient of variation *(PCV)* were computed as follow:

$$\text{GCV} = \sqrt{\frac{\textit{Genotypic variance}}{\textit{Grand mean of the trait}}} \text{ X } 100$$

$$\text{PCV} = \sqrt{\frac{\textit{Phenotypic variance}}{\textit{Grand mean of the trait}}} \text{ X } 100$$

Heritability in the broad sense (h^2) for each character was estimated as $h^2 = \delta^2_g / \delta^2_p$ (Singh & Chaudhary, 1985; Wricke & Weber,1986; Falconer & Mackay, 1996) and standard deviation of h^2 (sdh^2) was calculated as $\left[\frac{2}{N1+2} + \frac{2}{N2+2} \right] (1-h^2)$ where N_1 and N_2 are genotype and error degrees of freedom, respectively (Tenkouano et al., 2001). Expected genetic advance through selection which is also referred to as genetic gain (Burton & de Vane, 1953) or selection response (Wricke & Weber, 1986) is the difference between the mean of the progeny of selected individuals (X_p) and the base population (X_0) which is estimated as $GA = I \text{ x } h^2 \text{ x } p$ where I is the standardized selection differential assuming 5 % selection intensity and p is the standard deviation of δ^2_p.

Genetic variances from the combined data of the two locations were equated as: $\delta^2 g = (MS_g - MS_{gxl})/rn$ and $\delta^2_p = \delta^2 g + \delta^2_{gl}/n + \delta^2 e/rn$ where MS_{gxl} is mean square of the genotype x location interaction and n is the number of locations.

6.3.6. Genotypic and phenotypic correlations

Covariance analysis between all pairs of traits is a prerequisite in order to calculate the genotypic and phenotypic correlation coefficients. The covariances were calculated from the treatment sum of products and error sum of products and estimates of the genotypic and phenotypic covariances were calculated in the same manner as for the genotypic and phenotypic variance components. These covariance

components were used in the following formula to get the genotypic and phenotypic correlation coefficients as described by Miller et al. (1958).

$$\textit{Genotypic correlation coefficient} = \frac{Covx_1x_2}{\sqrt{(\delta^2 g_1)(\delta^2 g_2)}}$$

where Covx_1x_2 is the genetic covariance between two traits x_1 and x_2; δ^2_{g1} is the genotypic variance of the first character and δ^2_{g2} is the genotypic variance of the second character.

$$\textit{Phenotypic correlation coefficient} = \frac{Covx_1x_2}{\sqrt{(\delta^2 p_1)(\delta^2 p_2)}}$$

where δ^2_{P1} and δ^2_{P2} are the phenotypic variances of the first and second characters, respectively.

6.3.7. Path coefficient analysis

A path coefficient is a standardized partial regression coefficient and measures the direct influence of one variable upon another and allows the separation of the correlation coefficient into components of direct and indirect effects. The analysis was carried out as described by Dewy & Lu (1951), and Singh & Chaudhary (1985). Correlation coefficients between all possible pairs of the 10 characters were computed from the mean values of the two locations. The characters used were days to heading (DHE), days to maturity (DMA), plant height (PLH), spike length (SPL), number of seeds per spike (NS/SP), grain yield per spike (GY/SP), number of heads per square meter (NH/M^2), thousand kernel weight (TKW), grain filling period (GFP) and grain yield per plot (GY). Grain yield, being the complex outcome of the different characters, was considered as the resultant variable (effect) with the other characters as causal variables. Garcia del Morel et al. (1991) and Dofing (1997) explained an ontogenic approach that traits formed earlier in the ontogeny of the plant may have larger effects on latter developing traits. Accordingly in this study it is assumed that grain yield is a function of yield components such as NH/M^2, NS/SP, GY/SP and GFP. Similarly, GY/SP is supposed to be influenced by the earlier developed traits like NH/M^2 (basically tillers formed at an early stage of the development of the plants and reach to provide fertile heads), NS/SP and GFP. Hence path analysis was further computed to see the direct effects of the vegetative

period (DHE), NH/M^2 and GFP on NS/SP on one hand and the direct effects of NS/SP, NH/M^2 and GFP on GY/SP on the other.

The path analysis was done following the inverse matrix of the correlation matrix of characters from the combined data instead of for the individual locations because comparison of patterns of correlation matrices of the two locations using a Pearson product-moment correlation coefficient with NCSS 2000 program (Hintze, 1998) showed highly significant association (r=0.843) suggesting that the correlation matrices of the two environments vary in similar directions. Moreover, correlation values between characters in combined data had significant association with that of individual locations. Hence, combining the data from the two environments for the path analysis is feasible.

6.4. Results and discussion

6.4.1. Differences in agronomic traits

Analyses of variance for individual locations and combined over locations showed highly significant differences for all characters except for days to maturity in a combined analysis. The range of values and statistical significance for the different characters is presented in Table 6.1. Significant effects of testing locations were observed for grain yield per spike, number of heads per square meter, grain yield and 1000-kernel weight. Relatively higher number of heads per square meter was recorded at Sheno (394 ± 8.85) than at Keyit (300 ± 5.21). Grain yield and 1000-kernel weight were best expressed at Keyit, however. Interaction of location by landrace lines was observed only for days to heading and number of heads per square meter. The patterns of variation in the landraces for agronomic characters were similar across the two environments which indicate the consistency of their response to environmental variation for most characters (Figures 6.3A,B,C,D,E & F). Spearman rank correlation values based on the rankings of lines for each character at the two environments also revealed highly significant and positive associations between the rankings for all characters, despite the interaction of location with days to heading and number of heads per meter square, confirming the consistency of the relative performance of landrace lines at the two environments. The ranking of landrace lines

for grain yield at the two environments was different in some cases hence, the lower (r=0.67) rank correlation value (Figure 6.3D).

Combined over locations, highly significant (P=0.01) differences were observed among the landraces for all characters. Lines from Kessele, Tikur Gebs and Feres Gama and the standard check Misrach ranked top in grain yield. The analysis of variance within and between landraces illustrated apparent differences among landraces and to a certain extent within landraces. Among the landraces, lines from Feres Gama, Key Gebs, Bukura and Demoye demonstrated significant differences for most of the characters recorded at the two locations (Table 6.2). None of them, however, showed appreciable differences for grain yield.

Variation for the agronomic characters between lines from Kessele, Mage and Tikur Gebs were limited to a few of the characters observed (data not indicated). The former showed significant variation for days to heading and grain yield per spike at the two locations but only in one of the testing environments for days to maturity, plant height, spike length and number of seeds per spike. Lines from Mage were significantly different only in days to heading and maturity across environments and that of Tikur Gebs for 1000 kernel weight, number of seeds per spike and grain yield only.

Table 6.1.Range, mean and standard error (S.E), mean square for genotypes and level of significance of F-test for the different characters of barley landraces evaluated at two sites (S=Sheno & K=Keyit)

Characters	Site	Range	Mean ± S.E	Mean square	LSD	C.V (%)
Days to heading	S	73-97	84 ±0.37	76.49***	3.2	2.3
	K	72-98	81 ±0.29	78.52***	2.5	2.2
	S+K	62-94	81 ±0.26	165.55**	15.5	12.7
Days to maturity	S	117-150	129 ±0.55	156.04***	4.8	2.3
	K	117-144	127 ±0.42	174.56***	3.1	1.8
	S+K	119-143	124 ±0.37	546.12NS	NS	18.1
Plant height (cm)	S	67-122	102 ±0.67	79.90**	11.6	7.1
	K	68-117	99 ±0.45	111.06***	7.8	5.7
	S+K	80-110	100 ±0.40	182.36***	15.0	10.0
Spike length (cm)	S	3.5-10.4	7.3 ±0.09	5.01***	1.0	8.7
	K	3.9-10.1	7.0 ±0.08	5.57***	0.9	9.0
	S+K	4.2-9.3	6.9 ±0.07	9.12***	2.3	21.7
No. of seeds spike^{-1}	S	23-82	57 ±0.66	179.90***	9.2	9.8
	K	23-76	56 ±0.60	307.52***	7.4	9.5
	S+K	29-69	55 ±0.48	411.99***	18.0	21.8
Grain yield spike^{-1} (g)	S	0.98-4.1	2.3 ±0.05	0.824***	0.67	18.3
	K	1.4-4.8	2.8 ±0.04	1.13***	0.45	11.2
	S+K	1.6-3.6	2.5 ±0.03	1.45***	0.88	23.7
Number of heads/M^2	S	125-797	394 ±8.85	28232.30***	149.2	23.2
	K	122-562	300 ±5.21	14519.04***	89.2	21.3
	S+K	205-489	341 ±5.85	32433.67***	144.2	28.2
Grain yield plot^{-1} (g)	S	91-465	274.5 ±5.77	12207.85***	97.0	21.7
	K	194-1089	628.2 ±9.33	43216.17***	174.1	19.9
	S+K	289-622	441.6 ±10.64	35946.92***	177.4	26.8
1000 kernel weight (g)	S	23-52	37 ±0.41	73.32***	5.8	9.7
	K	31-61	46 ±0.31	85.86***	3.3	5.1
	S+K	31-52	40 ±0.35	111.59***	11.2	18.6
Biomass (g/plot)	K	240-2140	1199 ±18.29	148298.36***	353.0	21.1
Harvest index	K	0.28-0.68	0.47 ±0.00	0.006***	0.05	7.9

** significant at P < 0.01
***significant at P < 0.001

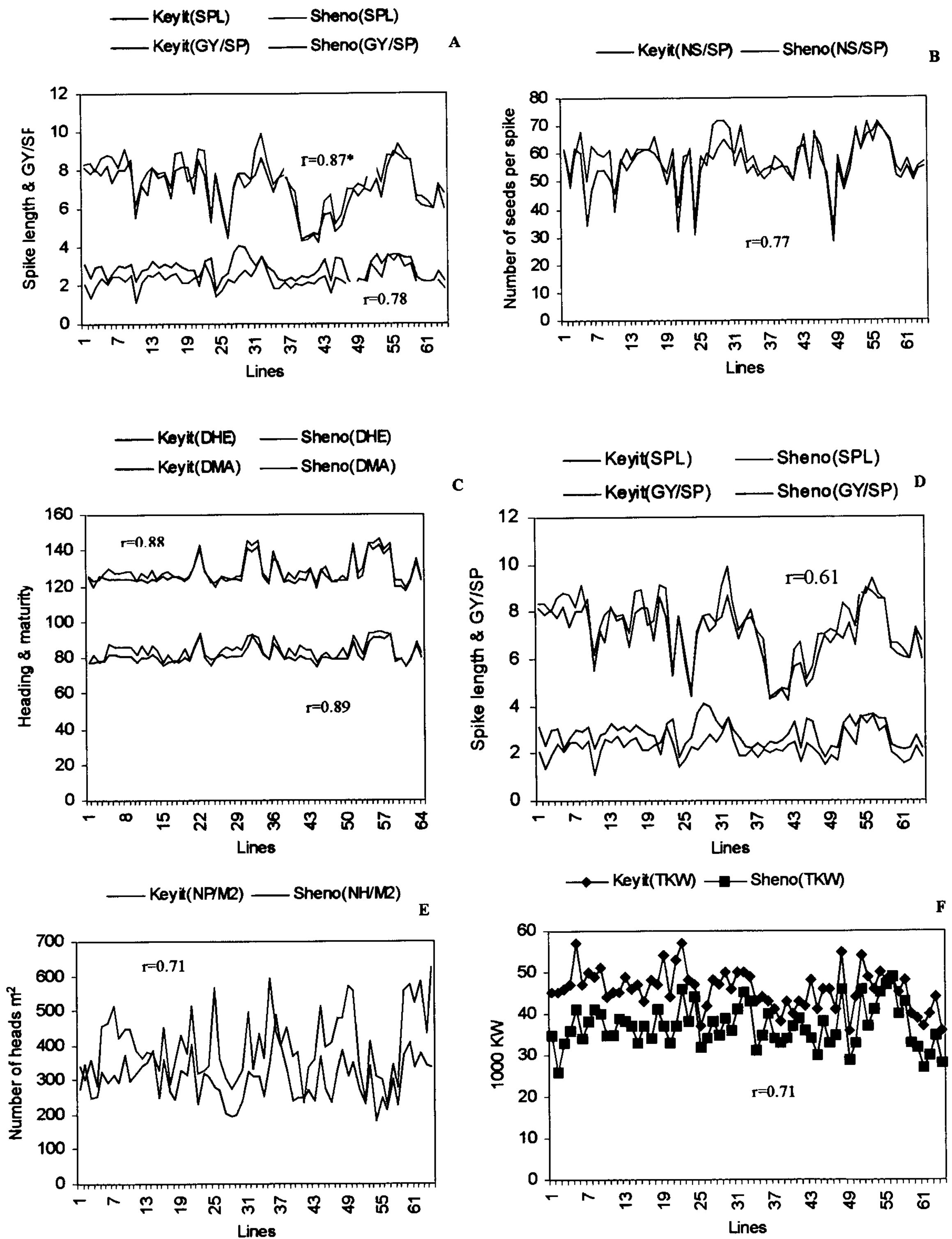

Figure 6.1. Patterns of variation for agronomic characters of landrace lines evaluated across environments (Keyit & Sheno) in 2001; SPL=spike length, GY/SP=grain yield spike^{-1}, NS/SP=number of seeds spike^{-1} DHE=days to heading, DMA=days to maturity and GY=grain yield. *=rank correlation values for the same characters at the two environments.

Table 6.2. Selections from landraces which showed significant differences for most of the agronomic characters across the two locations (S = Sheno & K = Keyit), 2001.

Landraces	DHE		DMA		PLH		SPL (cm)		NS/SP		GY/SP		NH/M^2		GY(g/plot)		TKW	
	S	K	S	K	S	K	S	K	S	K	S	K	S	K	S	K	S	K
F.Gama (22)*	94a	93a	143a	141a	99	87	9.0	7.8ab	59	55ab	3.2ab	3.3a	321	230	387	697	46a	57a
F.Gama (35)	92ab	86b	145a	141a	102	92	9.0	7.8ab	60	56ab	2.9ab	3.1ab	498	323	366	671	41a	50b
F.Gama (36)	93a	92a	143a	139a	103	94	9.9	8.7a	70	62a	3.5a	3.6a	331	312	395	719	45a	50b
F.Gama (37)	91b	87b	145a	142a	104	98	8.4	7.8ab	57	53b	2.6bc	3.0ab	434	311	407	746	43a	49b
F.Gama (38)	83c	82b	126b	127b	107	101	7.3	6.9b	59	56ab	1.9c	2.7b	365	252	258	559	31b	43c
LSD (5%)	2.3	4.9	6.2	3.6	NS	NS	NS	1.0	NS	7.9	0.86	0.4	NS	NS	NS	NS	6.8	2.4
C.V (%)	1.3	3.7	2.3	1.7	3.2	9.6	11.3	8.2	11.0	9.1	16.3	10.8	16.7	27.0	22.0	19.9	8.6	3.2
K.Gebs (24)	79b	76b	124ab	122	93b	94	7.8a	7.8a	31c	36d	1.4	1.8b	341b	308	184b	481	44a	47b
K.Gebs (25)	80b	80a	120c	122	102a	95	5.9b	6.3b	54ab	59a	1.7	2.4a	567a	278	331a	614	32bc	37e
K.Gebs (51)	79b	79a	122bc	122	91b	90	7.1a	6.2b	53ab	50bc	1.9	2.3a	477ab	332	311a	631	35b	41d
K.Gebs (52)	80b	79a	122bc	123	103a	99	7.0a	6.9ab	32c	28e	1.5	1.8b	477ab	387	292ab	557	46a	55a
K.Gebs (53)	84a	79a	126a	123	98ab	97	7.3a	6.7b	59a	56ab	1.9	2.3a	573a	312	324a	648	29c	36e
K.Gebs (54)	80b	79a	123abc	123	103a	99	7.1a	7.1ab	49b	47c	1.7	2.3a	557a	349	352a	599	33bc	44c
LSD (5%)	3.4	2.4	3.4	NS	8.2	NS	0.9	0.9	7.0	6.4	NS	0.4	176	NS	108	NS	4.6	3.2
C.V (%)	2.3	2.0	1.5	1.7	4.6	5.2	7.7	9.1	8.3	9.2	13.4	12.8	19.2	16.5	19.9	22.2	9.7	6.9
Bukura (23)	82a	80a	128a	130a	103	102a	5.4bc	5.3ab	60abc	62a	2.4a	3.5a	328b	320ab	247	547	38a	48a
Bukura (47)	84a	79a	130ab	129a	99	104a	6.3ab	5.7a	67ab	63a	2.5a	3.4a	357b	239b	248	581	34ab	47a
Bukura (48)	77b	75b	119c	121b	95	91b	6.7a	5.8a	51c	52b	1.6b	2.2b	516a	369a	294	614	30b	41b
Bukura (49)	81a	80a	128b	129a	105	97ab	5.3c	4.8b	68a	67a	2.4a	3.4a	396b	274ab	301	618	38a	46a
Bukura (50)	83a	80a	131a	126a	103	97ab	5.7bc	5.1ab	58bc	63a	2.2ab	3.4a	406ab	236b	240	600	33ab	46a
LSD (5%)	3.2	1.8	2.9	3.1	NS	8.2	0.9	0.7	10.1	7.3	0.73	0.2	121	99	NS	NS	6.8	2.5
C.V (%)	2.0	1.5	1.2	2.6	5.2	5.4	8.0	8.5	7.8	7.8	15.7	14.4	15.1	22.5	20.5	25.3	9.9	3.6
Demoye(1)	79b	77	127	125	92	98	8.3a	8.1a	62ab	62a	2.1a	3.1a	342	275	239	559	35a	45
Demoye(2)	82a	77	123	120	93	97	8.4a	7.9a	48bc	51bc	1.4b	2.4ab	300	344	142	658	26b	45
Demoye(3)	77b	78	124	126	104	102	8.0a	8.1a	59abc	62a	1.9a	2.9a	360	246	216	666	33a	46
Demoye(4)	79b	78	127	125	104	95	8.6a	7.8a	68a	60ab	2.4a	3.0a	284	253	234	591	36a	47
Demoye(10)	82a	77	123	123	98	93	6.2b	5.5b	39c	46c	1.1b	2.2b	447	298	142	553	35a	44
LSD (5%)	2.9	NS	NS	2.7	NS	NS	1.2	1.2	9.2	9.9	0.67	0.72	NS	NS	NS	NS	5.2	NS
C.V. (%)	1.9	1.9	2.2	1.5	5.3	4.8	7.5	10.6	20.8	11.5	15.6	17.2	28.3	24.6	28.4	17.0	8.4	5.8

*Figures in parenthesis refer to identification numbers given to lines. Means followed by the same letters in a column are not significantly different. DHE=days to heading, DMA=days to maturity, PLH=plant height, SPL=spike length, NS/SP=number of seeds spike^{-1}, GY/SP=grain yield spike^{-1}, NH/M^2=number of heads m^{-2}, TKW=1000-kernel weight

A comparison was made of the standard deviation (SD) for all landraces from combined data with the standard deviation of lines within each landrace according to Dierig et al. (1989) to assess variability for agronomic characters (Table 6.3) and support data in Table 6.2. The SD for all landraces was based on the 62 lines from 13 landraces and is indicated in bold below each character. Greater or comparable SD within a landrace to SD of all landraces indicates higher variability for the character considered and is indicated in bold. A relatively higher amount of variation was observed for days to heading, days to maturity and plant height within lines derived from Feres Gama. Bukura was variable for number of seeds per spike, grain yield per spike and 1000 kernel weight. Substantial variability existed within lines from Key Gebs for number of seeds per spike and 1000 kernel weight whereas differences within Nech Gebs were largely due to days to heading, 1000 kernel weight and grain filling period. This comparison is fairly consistent with the preceding explanation based on Table 6.2. Hence, results from the analyses of variance indicated in Table 6.1 reflect variations between landraces.

Table 6.3. A comparison of the standard deviation (SD) of characters of the lines derived from each of the landraces compared to the SD of the entire landraces (values in bold under the respective character). Standard deviation values comparable with SD of the entire landraces are indicated in bold.

Landrace	DHE	DMA	PLH	SPL	NS/SP	GY/SP	NP/M^2	GY	TKW	GFP
	5.16	**6.83**	**4.85**	**1.26**	**8.56**	**0.50**	**73.42**	**74.58**	**4.49**	**7.08**
1153	1.14	0.55	2.41	0.30	1.87	0.21	16.84	14.54	1.34	-0.59
1182	1.82	1.48	3.05	0.15	3.70	0.13	21.18	22.38	1.14	0.84
Bukura	2.39	3.67	3.78	0.72	**9.13**	**0.48**	42.16	44.65	**5.24**	1.79
Demoye	1.00	2.39	3.08	0.99	6.52	0.32	43.76	28.47	3.19	3.27
Feres Gama	**8.08**	**6.25**	**4.83**	1.02	6.88	**0.49**	51.43	68.68	**6.22**	**9.61**
Feleme	**5.12**	6.00	**7.29**	0.52	4.93	0.27	47.13	36.22	3.21	**7.19**
Key Ferke	1.48	0.84	4.56	0.74	2.07	0.12	61.59	70.33	1.52	2.24
Key Gebs	1.64	0.55	4.21	0.58	**11.78**	0.23	68.03	62.84	**6.55**	1.41
Kessele	1.58	0.84	3.03	0.64	6.62	0.28	12.67	51.28	2.07	0.84
Mage	1.92	1.73	1.67	0.19	2.30	0.10	40.79	46.46	1.64	0.84
Nech Gebs	**13.48**	2.39	3.63	0.50	3.58	0.19	41.08	62.69	4.32	**14.36**
Tikur Gebs	0.55	0.55	3.13	0.92	7.33	0.28	44.08	68.06	2.59	0.71

6.4.2. Principal component analysis

The important principal components, the percentage of the total variance that each represents, and the coefficients used in the weighted sum (loadings) are presented in Table 6.4. The first four principal components (PC) explained 83.23 % of the variation of landraces evaluated at Sheno, 84.44 % at Keyit, and 81.28 % in combined data. Characters that contributed more to the first principal component (variables with largest coefficients) are days to maturity, 1000-kernel weight, days to heading, grain filling period, grain yield per spike and spike length at Sheno; days to maturity, grain filling period, days to heading and plant height at Keyit; grain filling period, days to maturity and plant height when data were combined from the two locations. The contribution of these characters was negative except plant height from data across environments. The second component illustrated primarily the variation in number of heads per square meter and grain yield per plot at Sheno; number of seeds per spike, grain yield per spike and number of heads per meter square at Keyit; 1000- kernel weight, days to heading, spike length, days to maturity and grain yield per spike when Sheno and Keyit were combined. The third principal component separated landraces only with number of seeds per spike at Sheno; spike length at Keyit and with grain yield and number of heads per square meter for the combined data. The fourth principal component described the variation in plant height at Sheno, grain yield per plant at Keyit; number of seeds per spike, grain yield per spike and number of heads per square meter for the combined data. Generally, it indicates that the landraces with high PC1 values are low yielding ones characterized by low 1000-kernel weight, relatively short grain filling duration, comparatively short spike length and early in heading and maturity. Those landraces with high PC2 values had high grain yield characterized by high number of heads per square meter across environments except in a combined data. In most of the cases, the contribution of number of heads per square meter to each PC is in the opposite direction to the contribution made by number of seeds per spike and grain yield per spike (Table 6.4) indicating the need to consider the relationships of these yield components in a selection program.

Table 6.4. Eigenvalues, proportion of the total variance and contribution of agronomic characters to the first four principal components of barley landraces evaluated at Sheno and Keyit in Ethiopia, 2001.

Characters	Sheno				Keyit				Combined			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
Eigenvalue	3.88	1.45	1.77	1.22	2.64	2.35	1.88	1.56	2.01	2.31	1.61	2.19
Proportion of δ^2	38.77	14.50	17.74	12.23	26.39	23.56	18.84	15.64	20.15	23.12	16.10	21.91
Cumulative δ^2	38.77	53.27	71.00	83.23	26.39	49.95	68.80	84.44	20.15	43.27	59.37	81.28
Factor loadings												
Days to heading	-0.781	-0.050	-0.183	0.393	-0.411	0.129	-0.219	-0.032	0.388	-0.663	0.140	0.198
Days to maturity	-0.916	0.013	-0.270	0.039	-0.451	0.083	-0.333	0.031	-0.686	-0.494	0.142	0.317
Plant height	-0.133	0.200	-0.085	0.871	0.025	0.189	0.618	-0.104	0.602	-0.284	0.245	0.284
Spike length	-0.607	0.183	-0.151	-0.007	-0.303	0.377	0.171	-0.071	-0.250	-0.571	0.394	0.218
No seeds spike^{-1}	-0.128	-0.035	-0.979	0.030	-0.278	-0.424	0.262	0.452	0.012	0.043	0.027	0.974
G. yield spike^{-1}	-0.679	-0.058	-0.661	0.159	-0.405	-0.251	0.348	0.097	-0.159	-0.475	-0.058	0.839
No plants m^{-2}	0.374	0.818	0.353	0.046	0.236	0.521	-0.148	0.324	0.223	0.283	0.744	-0.475
Grain yield plot^{-1}	-0.366	0.816	-0.197	0.223	-0.143	0.466	0.221	0.538	-0.041	-0.285	0.892	0.129
1000 kernel wt.	-0.879	-0.153	0.037	0.158	-0.306	0.257	0.173	-0.602	-0.016	-0.912	-0.031	0.010
Grain filling period	-0.718	0.093	-0.275	-0.452	-0.349	-0.007	-0.371	0.105	-0.944	0.007	0.034	0.161

6.4.3. Cluster analysis

Summary of the agronomic characters of landraces within a cluster and deviations of means for grain yield among clusters are presented in Tables 6.5 and 6.6, respectively. Clustering of landraces based on data from each location were very similar to that resulting from combined data except for few lines which did not follow the same pattern (Figures 6.1, 6.2 & 6.3). Hence, discussion was based on data combined from the two locations. The genetic relationship as revealed by the hierarchical cluster analysis explicitly showed tight grouping of lines derived from landraces bearing the same name. This verified the predominance of variation among landraces rather than within landraces. Cluster I, for instance, comprised all the lines from landrace 1153 and four of the five lines from Demoye and Key Ferke each forming distinct subgroups within the cluster. Three lines from Bukura also appeared in this cluster. Two of the five lines from Bukura did not appear together with their corresponding lines in this cluster because they had a significantly lower number of seeds per spike, harvest index and 1000-kernel weight. Two lines from Feleme and one from Feres Gama appeared in cluster II. Three of the lines from the former landrace were excluded from this cluster because of significant differences in days to heading, days to maturity, grain yield per spike and plant height. This was also apparent from the analysis of variance in combined data. Lines in this cluster had

high grain yield and biomass such as those in cluster III but were late in heading and maturity.

All lines from Kessele and all except one from Tikur Gebs, including the check variety Misrach, appeared in cluster III. These lines were as early as those in cluster I and IV, but had better grain yield potential than lines in the latter two clusters (Table 6.5). Cluster IV comprised those which had less grain yield per plot, grain yield per spike, biomass, number of seeds per spike, and 1000-kernel weight than their corresponding lines from Bukura and Demoye in cluster I. A high number of plants per unit area was observed from landrace lines in cluster V and it included all Mage and Key Gebs selections while cluster VI had only lines from landrace 1182 which had very short and compact spikes. It was not possible to distinguish in the field the lines derived from Feres Gama and Nech Gebs. However, only two lines from Feres Gama and three lines from Nech Gebs appeared in the same cluster while the rest appeared separately on their own.

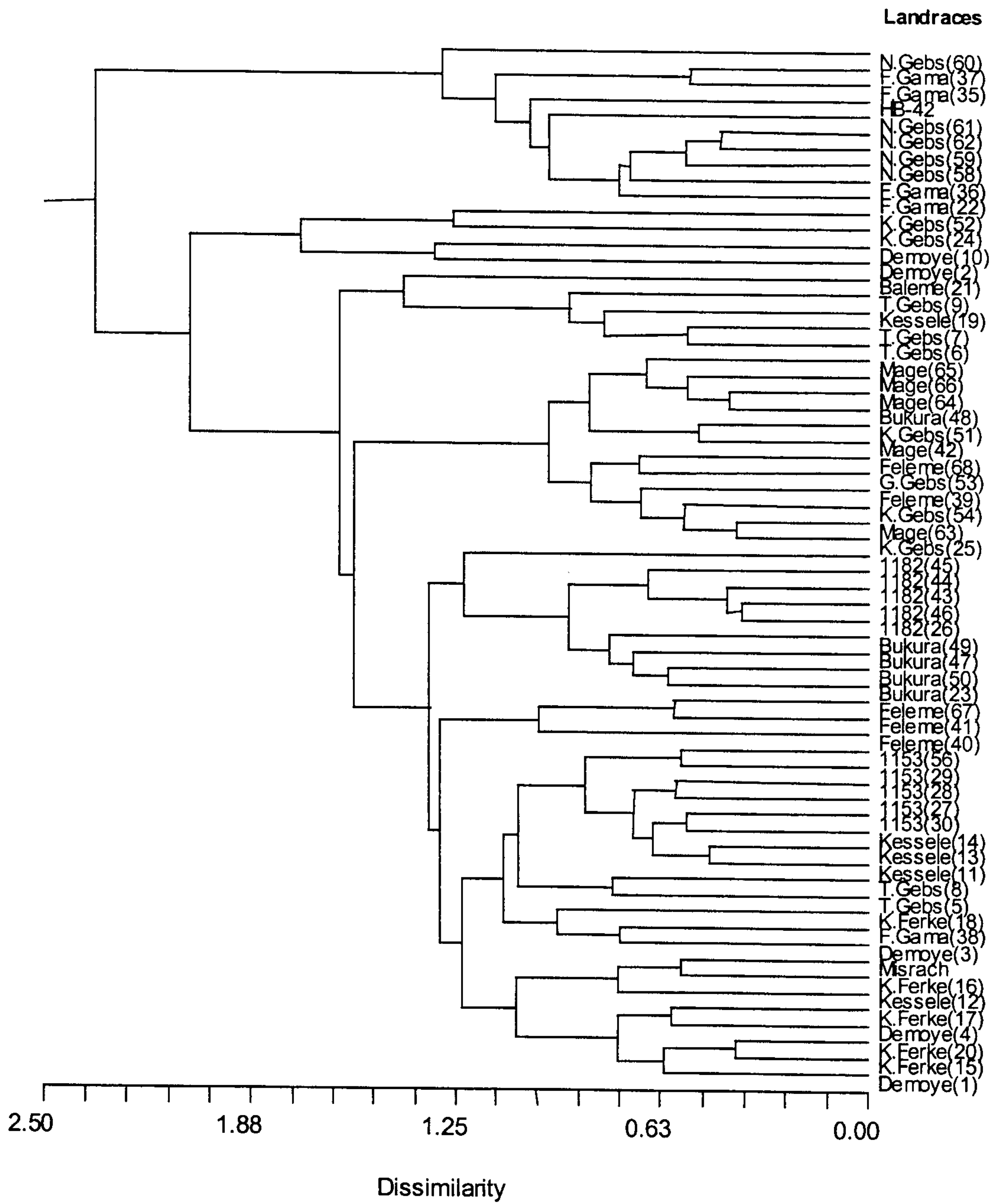

Figure 6.2. Genetic relationships between barley landrace lines from north Shewa in Ethiopia evaluated for quantitative characters at Sheno in 2001.

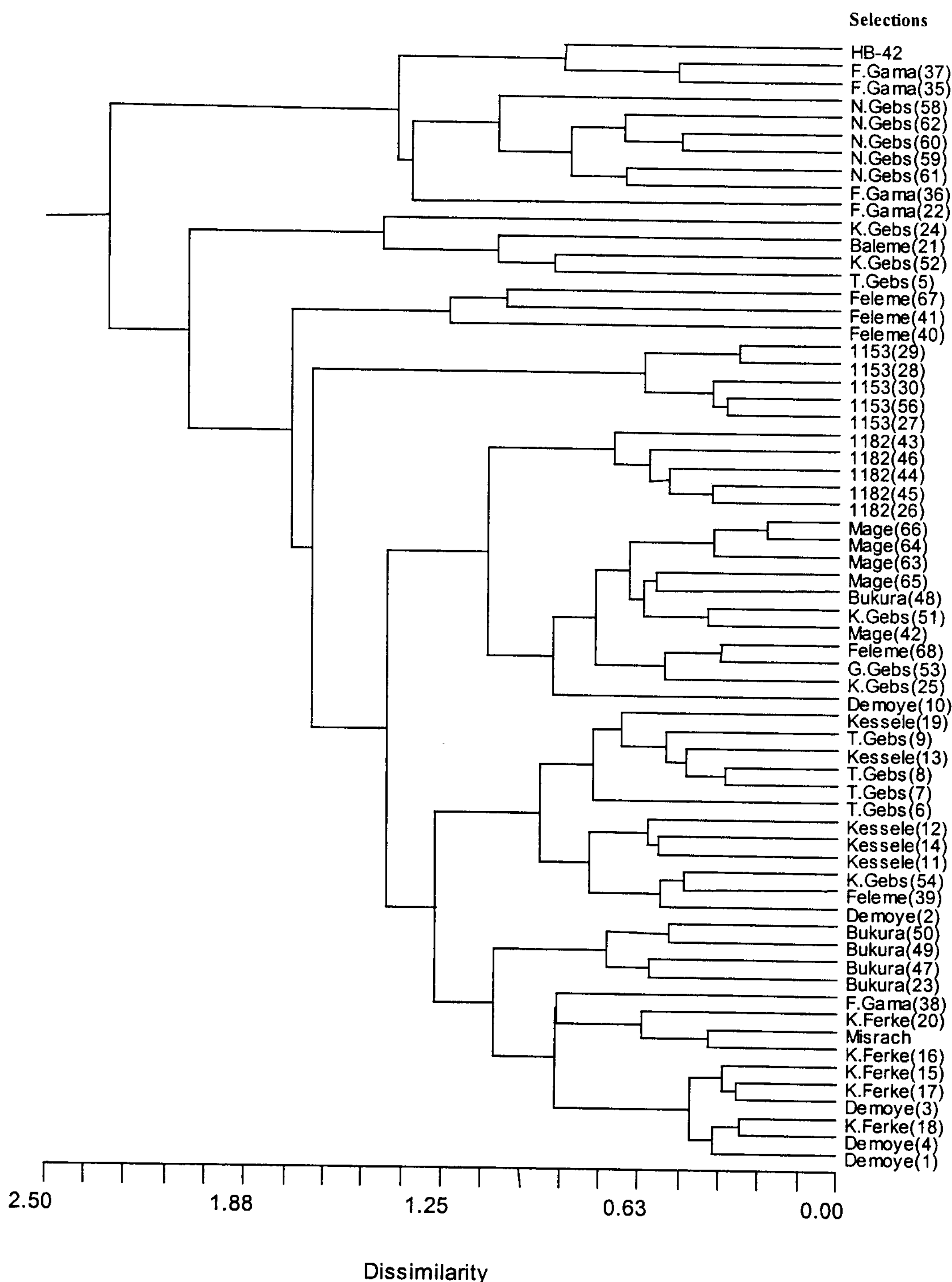

Figure 6.3. Genetic relationships between barley landrace lines from north Shewa in Ethiopia evaluated for quantitative characters at Keyit in 2001.

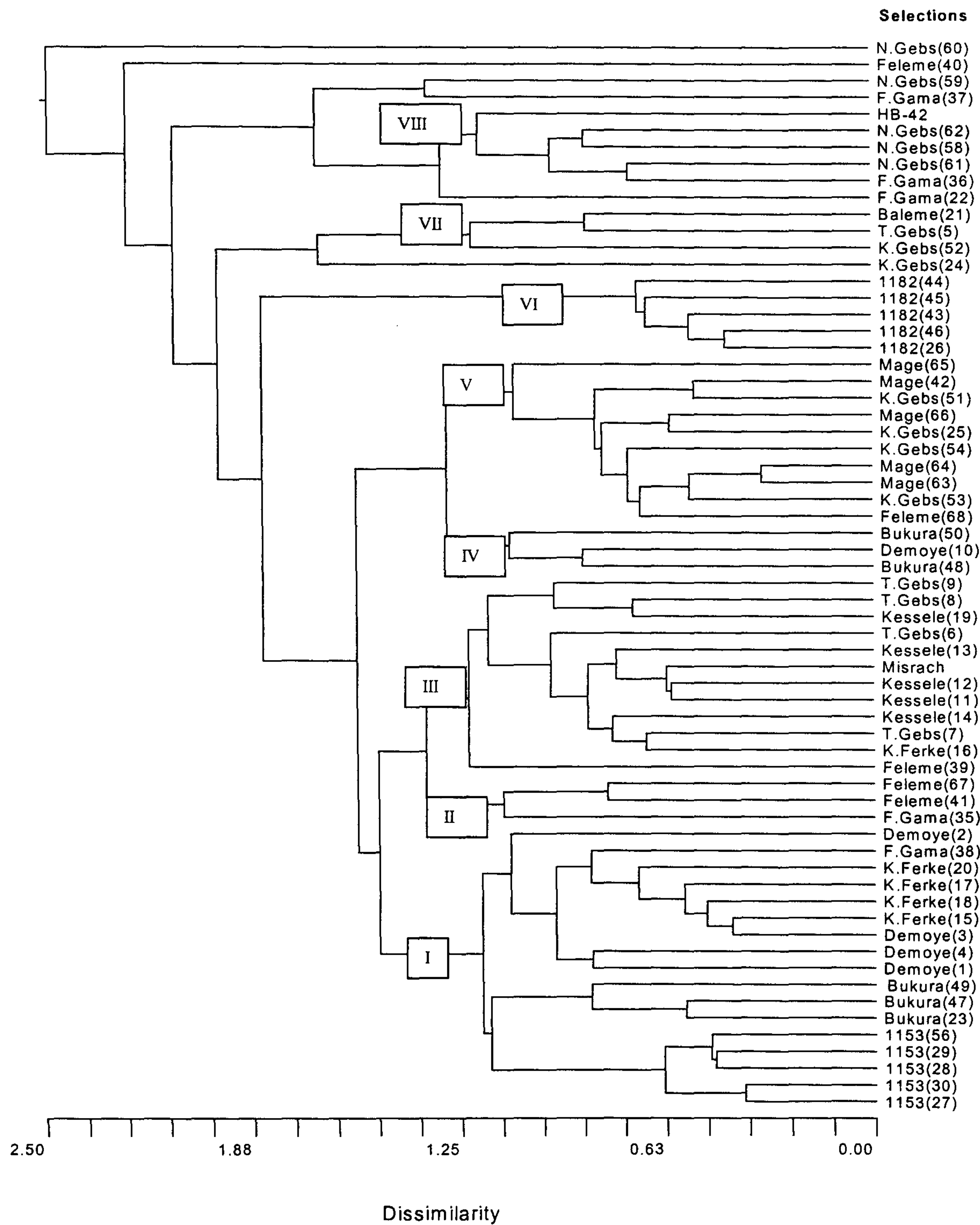

Figure 6.4. Dendrogram illustrating the genetic relationships between barley landrace lines from north Shewa in Ethiopia evaluated for quantitative agronomic characters at Sheno and Keyit in 2001.

Table 6.5. Intra-cluster mean and standard deviation values of agronomic characters of lines from combined data of Sheno and Keyit, 2001.

Cluster	DHE	DMA	PLH	SPL	NS/SP	GY/SP	NH/M^2	GY	BM	HI	TKW
I	81+2.6	125+2.5	101+3.8	7.1+1.11	61+5.9	2.7+0.41	289+34	407.7+29.1	1099.1+78.9	0.48+0.02	41
II	88+1.5	136+4.2	99+4.2	7.1+0.26	53+4.9	2.4+0.17	401+6	516.6+8.3	1322.3+131.5	0.45+0.02	40
III	83+1.9	124+1.7	105+3.3	7.3+0.64	54+6.7	2.5+0.27	383+41	527.5+50.9	1426.7+142.4	0.48+0.03	42
IV	79+2.6	123+2.5	96+3.1	5.1+0.65	44+4.0	1.9+0.35	317+63	376.5+20.1	1015.0+67.3	0.49+0.02	35
V	79+1.9	122+1.3	97+3.1	6.4+0.34	54+2.7	2.0+0.10	446+33	464.0+37.2	1159.5+91.8	0.49+0.03	35
VI	82+1.8	123+1.5	100+3.0	4.5+0.15	56+3.7	2.4+0.13	297+21	315.9+22	921.0+94.0	0.39+0.01	38
VII	84+3.2	126+3.8	105+3.5	8.1+1.00	36+6.6	2.0+0.31	429+29	438+15	1305.0+117.6	0.41+0.03	48
VIII	92+0.5	141+2.6	100+3.1	8.8+0.36	67+2.2	3.4+0.18	281+44	511+39	1341.0+122.8	0.47+0.01	44

Note: abbreviations for the characters are as described for Table 5.2.

The intracluster mean values for grain yield were very high and comparable for clusters II, III and VIII whereas the lowest mean was observed in cluster VI (all derived from acc.1182). Accordingly mean deviations for grain yield between this cluster and clusters II, III and VIII were very high. Landrace lines with comparable and superior agronomic performance but genetically dissimilar can exhibit heterosis in yield and the hybrids generated would be similar to their parents and equally adaptable. Moreover, landraces divergent for quantitative characters can be used for recombination in breeding programs since highly divergent landraces would perform a wide spectrum of variability enabling further selection and improvement. Landraces in clusters II, III and VIII, for instance, are comparable in grain yield and superior to landraces in other clusters. However, landraces in cluster VIII are distinct (Table 6.5 & Figure 6.5) to those in clusters II and III in days to heading, maturity, and spike length, number of seeds per spike and grain yield per spike. Hence, crossing among these clusters is supposed to combine characters of economic importance without affecting the final grain yield. Cox et al. (1985) suggested crossing distantly related lines in an inbred improvement program to maximize the number of loci segregating in the F2 and subsequent inbred generations and exploit higher variances for quantitatively inherited characters in segregating populations.

The overall genetic relationships between the landraces were also investigated based on the mean performance pooled from the lines they constitute (Figure 6.5). Cluster

position of landraces in the dendrogram corresponds well with grouping based on visual assessment and is a perfect reflection of the morphological and phenological similarities. Farmers often describe the similarities between Feleme and Feres Gama. The presence of Feleme with Mage and Key Gebs in cluster II is, therefore, opposite with their traditional classification. Farmers often exchange seeds from within or outside their localities. Consequently, the newly introduced landrace to that locality may be given a similar name to the landrace they knew before, if it resembles one of the morphological features; seed colour and spike length for example. In such a way the farmer who was the source of seed of Feleme might have given it the wrong name, hence the two landraces (Feleme and Feres Gama) supposed to be clustered tightly appeared in different clusters. This landrace was looked upon with suspicion while evaluating the materials under field condition because it was early in heading and maturity compared to Feres Gama. Informal discussion with a local farmer familiar with these landraces also expressed his feeling that Feleme is not what it is supposed to be.

Table 6.6. Cluster means (bold) and deviations of means among clusters for grain yield of barley landraces evaluated at Keyit and Sheno in Ethiopia.

Cluster	I	II	III	IV	V	VI	VII	VIII
I	**407.7**	108.3	119.8	31.2	56.3	91.8	30.3	103.3
II		**516.6**	10.9	140.1	52.6	200.7	78.6	5.6
III			**527.5**	151.0	63.5	211.6	89.5	16.5
IV				**376.5**	87.5	60.6	61.5	134.5
V					**464.0**	148.1	26.0	47.0
VI						**315.9**	122.1	195.1
VII							**438.0**	73.0
VIII								**511.0**

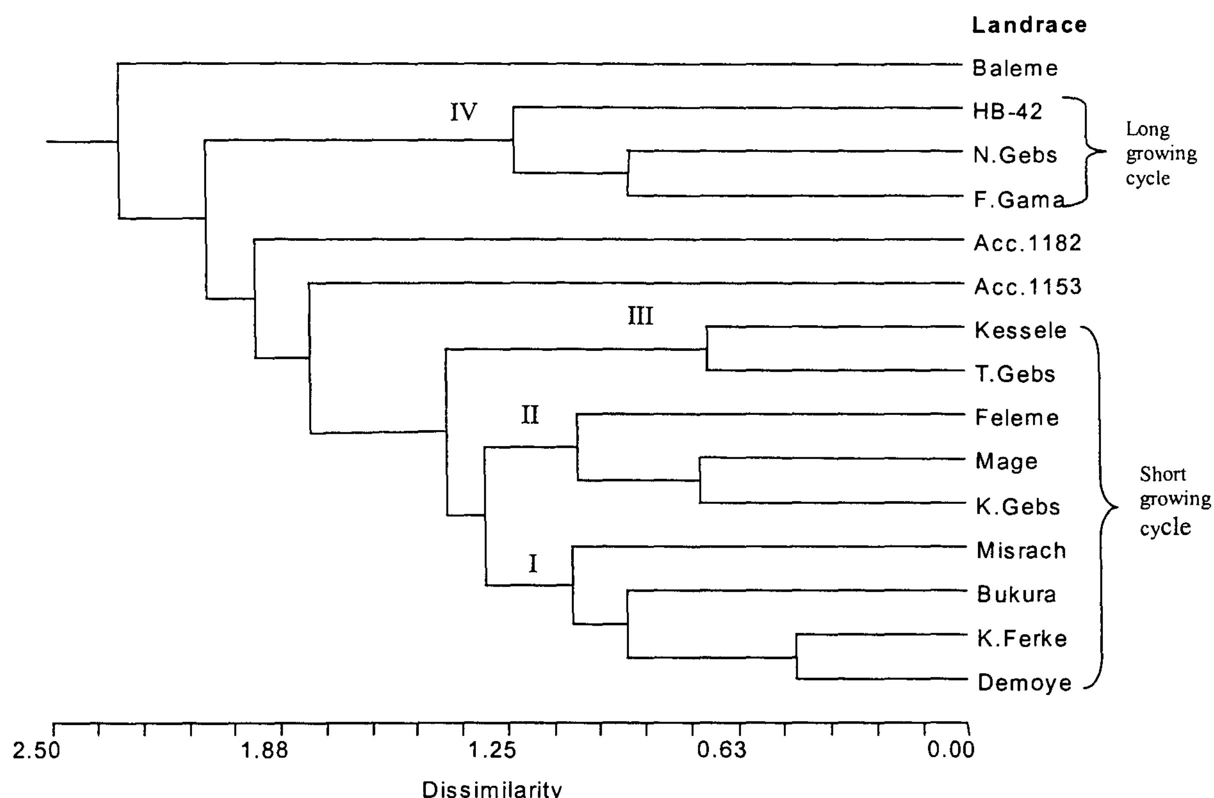

Figure 6.5. Genetic relationship between farmers' barley cultivars predominantly grown in North Shewa, Ethiopia based on quantitative morphological characters. Acc.1182 and Acc.1553 are landrace collections.

6.4.4. Estimates of genetic variances and implications for improvement

Phenotypic (PCV), and genotypic (GCV) coefficient of variation, estimates of the components of variances, broad sense heritability (H), expected genetic advance and genetic advance as percent of the mean are presented in Tables 6.7 and 6.8. The genetic variance values indicate the genetic variability of landraces and the potential advance that may be made for each character selected. Phenotypic variances for number of heads per unit area and grain yield per plot are far greater than the respective genotypic variances. The two types of variances are very close for the rest of the characters, however. The magnitude of the variance components at the two locations and the differences between variance components at the two locations were not substantial. This was not the case for the combined estimates in that the phenotypic variances were greater than the genotypic variances.

The ranges of values of the genetic variances mean little unless the unit of measurement and the mean is known (Burton & De Vane, 1953). The relative amount of genetic variation is best expressed as the genotypic coefficient of variation since this variable also takes the mean value and the unit of measurement into consideration. Relatively higher PCV than GCV values were observed for all the characters but in most cases the two values differed slightly, indicating small environmental effects in estimating these parameters. Number of heads per unit area and grain yield per spike displayed the highest PCV values at each location and across locations. PCV value for grain yield was higher at Sheno and across environments but less at Keyit. Spike length, number of seeds per spike and biomass had moderate values of PCV. The trend was similar for GCV estimated for individual environments and across environments (Table 6.5 & 6.6). GCV ranged from very low (2.98) for plant height to 20.5 for grain yield per spike at Sheno and from 5.12 for days to maturity to 17.91 for grain yield per spike at Keyit. Grain yield per spike generally attained the maximum GCV values as estimated for single environments and across environments. Moreover, the reproductive characters (spike length, grain yield per spike and number of seeds per spike) generally had considerably higher GCV values than the vegetative characters such as days to heading, days to maturity and plant height. This corroborates with Alemu (2001) who reported higher values of GCV for spike length, number of seeds per spike and grain yield per spike. Such high relative GCV values for reproductive characters is also consistent with the results of several studies in grasses suggesting reproductive characters are more variable than vegetative ones (Sachs & Coulman, 1983; Rognli, 1987; Finne et al., 2000).

Differences in GCV values of the two environments are attributed to differences in the mean values of the characters at the respective locations. GCV and genotypic variances from individual locations are higher compared to those estimated across environments. Variances estimated in single environments are generally expected to be biased upward due to confounding of genotype x location interaction effect (Wricke & Weber, 1986; Fehr, 1987; Finne et al., 2000). Moreover, in this experiment only a one-year test was done and δ^2gy and δ^2gly cannot be estimated

and are confounded with δ^2g and δ^2gl hence higher values in the latter two variances is expected (Wricke & Weber, 1986; Fehr, 1987).

Broad sense heritability values were very high for all characters at the two locations except for plant height and biomass. Broad sense heritability values from combined estimates were observed to be less in magnitude than from individual locations because the upward biased values in the latter case due to confounding effect of δ^2gl were removed when data were combined. Heritability for days to heading in a combined estimate was negative because of the negative estimate of genotypic variance. Negative estimates of the variance components shall be assumed zero (Rasmusson & Glass, 1967). However, in order to obtain unbiased estimates of phenotypic variance, the negative variance component estimates shall be treated as negative values when computing the phenotypic variances, which in turn are used in computing expected genetic advance (Miller et al., 1958). Accordingly, the negative value of heritability for days to heading was considered as zero. Low heritability may arise as a result of poor adaptability (Finne et al., 2000) but these materials were not poorly adapted and there was no obvious explanation for such low estimate for days to heading when data were combined. Days to maturity, spike length, and number of seeds per spike had the highest values in each environment and across environments. Grain yield and plant height had comparatively low values in all cases indicating the influence of environment on these characters. Often characters like yield are much influenced by environmental effects and their heritability is therefore low (Wricke & Weber, 1986).

Genetic advance

The use of heritability estimates in conjunction with selection differential (Rasmusson & Glass, 1967), and heritability accompanied with a good level of GCV (Burton & De Vane, 1952) to predict advance from selection for testing programs is essential. The heritability estimates that were obtained in self fertilizing crops may result in inflated estimates of genetic advance; however, this may not be a serious problem if recognition is given to limitations associated with the heritability estimates employed to predict advance from selection (Rasmusson & Glass, 1967). Testing genotypes with more replications, locations and years will increase accuracy

of prediction of advance from selection. However, it is emphasised that there is little advantage in testing for two years at one location as compared to two locations in a single year (Rasmusson & Glass, 1967). Therefore, from this experiment it is possible to infer selection advance that may be achieved especially considering estimates from combined data where the confounding effect of δ^2gl was excluded.

Table 6.7. Phenotypic (PCV) and genotypic (GCV) coefficient of variation, components of variances, broad sense heritability (H) and expected genetic advance from selection (GA) of 11 characters of barley landrace lines evaluated at Keyit (K) and Sheno (S) in Ethiopia, 2001.

Characters	Location	PCV (%)	GCV(%)	δ^2p	δ^2g	H	GA	GA (% mean)
Days to heading	K	5.46	5.35	19.63	18.83	0.96	8.76	10.79
	S	5.99	3.85	25.50	24.23	0.95	9.88	11.74
Days to maturity	K	5.20	5.12	43.64	42.37	0.97	13.20	10.39
	S	5.60	5.44	52.01	49.06	0.94	13.96	10.84
Plant height	K	5.32	4.49	27.77	19.75	0.71	7.71	7.79
	S	5.06	2.98	26.63	9.29	0.35	3.72	3.65
Spike length	K	16.75	16.14	1.39	1.29	0.93	2.26	32.12
	S	17.54	16.80	1.67	1.53	0.92	2.45	33.23
No of seeds/spike	K	15.58	14.84	76.88	69.79	0.91	16.44	29.21
	S	13.45	12.18	59.97	49.18	0.82	13.08	22.72
Grain yield/spike	K	18.92	17.91	0.29	0.26	0.89	0.99	34.77
	S	23.08	20.50	0.28	0.22	0.78	0.84	37.10
No. of heads/M^2	K	20.05	16.99	3629.76	2606.27	0.72	89.36	29.74
	S	24.42	20.40	9410.77	6567.33	0.69	137.89	34.71
Grain yield/plot	K	16.53	13.23	10804.05	6904.98	0.64	137.04	21.79
	S	22.17	18.30	3769.28	2567.55	0.70	88.53	31.97
Biomass	K	16.06	12.10	37074.59	21050.09	0.57	226.00	18.86
Harvest index	K	8.51	7.67	0.0016	0.001	0.81	0.00	14.26
1000 kernel weight	K	10.15	9.82	221.47	20.10	0.94	8.97	19.65
	S	6.44	12.22	24.44	20.17	0.83	8.45	11.01

Table 6.8. Phenotypic (PCV) and genotypic (GCV) coefficient of variation, components of variances, broad sense heritability (H) and expected genetic advance from selection (GA) from combined data of 11 characters of barley landrace lines evaluated at Keyit and Sheno, Ethiopia 2001.

Characters	PCV (%)	GCV(%)	δ^2p	δ^2g	H $\pm$SE	GA	GA (% mean)
Days to heading	8.63	-0.91	49.50	-0.55	-1.00	-0.15	-0.18
Days to maturity	5.22	4.41	42.30	30.51	0.72$\pm$0.02	9.64	7.74
Plant height	5.41	3.77	29.26	14.21	0.49$\pm$0.03	5.46	5.47
Spike length	17.13	15.79	1.41	1.20	0.85$\pm$0.01	2.08	30.02
Number of seeds/spike	14.63	12.43	64.60	46.66	0.72$\pm$0.02	11.92	21.69
Grain yield/spike	20.47	17.04	0.26	0.18	0.69$\pm$0.02	0.73	29.48
No. of plants/M^2	23.58	16.93	6463.65	3332.29	0.52$\pm$0.03	86.12	25.26
Grain yield/plot	18.57	12.98	6727.16	3284.95	0.49$\pm$0.03	82.79	18.75
1000 kernel weight	10.27	7.94	16.95	10.14	0.59$\pm$0.02	5.00	12.47

Among the characters with high heritability, only number of seeds per spike, spike length, number of heads per square meter, and grain yield per spike combined higher values of GCV and heritability. Accordingly, these three characters had higher values of genetic advance expressed as a percentage of the mean with values of 21.7 % for the former and 30 % for the latter two. Days to maturity and number of seeds per spike had equal heritability values, but because of differences in the size of the GCV, the expected genetic advance for days to maturity was very low when compared to that of GCV for number of seeds per spike (Table 5.6). This demonstrates the effect of GCV on genetic advance when heritability values are similar.

The level of variability within landraces and among lines from different landraces was further assessed by means of variance estimation on cluster basis from Figure 5.4. Three dominant clusters (CI, CIII, and CV) which, together, comprised more than half of the lines evaluated were taken as an example to illustrate within cluster variability of landraces for agronomic characters and genetic advance that may be expected. The variance components, GCV, PCV, and expected gain from selection of lines within clusters were lower (Table 6.9) than the corresponding values observed for all landrace lines (Table 6.8) confirming higher variability for agronomic characters among landraces than within landraces. Only GA (% of mean) for spike

length and grain yield per spike in cluster I were significant, however, because this cluster comprised three distinct sub groups differing in spike length (eg. Bukura with short spike length) and those with long spikes (acc.1153, Demoye, Key Ferke) but all were early.

Table 6.9. Estimates of genetic variances and expected genetic advance of lines within clusters

Cluster	Trait	Mean	δ^2g	δ^2gl	δ^2p	GCV	PCV	H	GA	GA (%)
I	DHE	81	9.49	5.65	22.66	3.85	5.95	0.42	4.11	5.14
	DMA	125	3.92	-30.10	45.85	1.59	5.46	0.09	1.19	0.96
	PLH	101	13.28	-8.89	30.27	3.61	5.45	0.44	4.97	4.92
	SPL	7.1	0.95	-0.31	1.17	13.75	15.25	0.81	1.81	25.52
	NS/SP	61	14.77	-26.11	29.87	6.40	9.11	0.49	5.57	9.28
	GY/SP	2.7	0.11	0.00	0.16	12.30	14.94	0.68	0.56	20.87
	NP/M2	289	333.98	-772.3	1061.4	6.30	11.23	0.31	21.12	7.28
	GY	407.7	-40.42	-2465	450.11	-1.558	5.20	-0.09	-3.93	-0.96
	TKW	40	4.14	1.38	8.47	5.09	7.27	0.49	2.93	7.33
III	DHE	83	-1.04	-7.05	29.26	-1.27	6.76	-0.04	-0.39	-0.49
	DMA	124	-6.20	302.73	229.05	-2.09	12.72	-0.03	-0.84	-0.71
	PLH	105	-1.39	-39.42	36.95	-1.16	5.96	-0.04	-0.47	-0.47
	SPL	7.3	-0.38	1.64	1.02	-8.56	14.02	-0.62	-0.77	-10.75
	NS/SP	54	12.49	60.27	72.78	6.67	16.07	0.17	3.02	5.69
	GY/SP	2.5	0.03	0.08	0.15	7.05	15.61	0.20	0.16	6.56
	NP/M2	383	987.67	2915.1	4759.4	8.45	18.55	0.21	29.49	7.93
	GY	527.5	-78.95	1054.6	3028.9	-1.68	10.44	-0.03	-2.96	-0.56
	TKW	41	-2.57	-8.08	4.37	-3.90	5.09	-0.59	-2.53	-6.18
V	DHE	79	2.55	1.49	3.75	2.02	2.45	0.68	2.71	3.43
	DMA	122	2.19	0.69	3.11	1.21	1.45	0.71	2.56	2.10
	PLH	97	8.19	-2.87	10.62	2.95	3.36	0.77	5.18	5.34
	SPL	6.4	0.09	-0.04	0.12	4.68	5.51	0.72	0.53	8.20
	NS/SP	54	7.77	-2.91	9.17	5.26	5.71	0.85	5.29	9.97
	GY/SP	2.0	0.00	0.00	0.01	2.98	5.01	0.35	0.07	3.66
	NP/M2	446	809.81	-1237	1491.1	6.31	8.56	0.54	43.20	9.58
	GY	464	974.45	-995.3	1516.9	6.71	8.37	0.64	51.54	11.08
	TKW	36	9.73	-2.50	13.87	8.66	10.35	0.70	5.38	14.95

6.4.5. Genotypic and phenotypic correlations

Genotypic and phenotypic correlations from data at Keyit and Sheno are shown in Tables 6.10 and 6.11, respectively and in Table 6.12 for data across the environments. Although the genotypic correlation values were slightly higher than the phenotypic correlation values at the two locations, in a number of cases the

genotypic and phenotypic correlation coefficients were very close in magnitude suggesting the environmental variances and covariances were very small and the influence of environment on these relationships was minimal (Falconer, 1989) or the environmental variances and covariances had been reduced to zero or to a negligible level (Estilai et al., 1992). The direction of association (negative or positive signs of correlation coefficients) and the level of statistical significance of correlation coefficients among the different pairs of characters followed more or less similar trends both at the phenotypic and genotypic level at both locations. The exception is number of heads per unit area which had no significant association with days to heading and days to maturity at Keyit while the corresponding phenotypic and genotypic correlation coefficient values at Sheno were negative and highly significant. Hence, throughout the remainder of this section only the genotypic correlation coefficient is discussed.

Number of heads per unit area has negative correlations with all characters except with grain yield per plot, plant height and biomass. Grain yield was strongly and positively associated with plant height, spike length, number of heads per unit area and harvest index at both locations. Harvest index, which was highly and positively correlated with grain yield, had highly significant and positive association with number of seeds per spike also. Number of seeds per spike had positive but a very weak association with grain yield, however. Grain yield per spike was strongly and positively associated with days to heading, days to maturity, plant height, spike length, number of seeds per spike, grain yield per plot. Generally, among the yield components, days to heading, days to maturity, spike length, number of seeds per spike, grain yield per spike and grain yield per plot showed the expected positive correlations (except number of seeds per spike vs grain yield per plot) indicating the potential of improving yield through selection for these characters. The positive and significant association of grain yield with grain yield per spike, biomass and harvest index corroborates with results of Alemu (2001). Similar patterns of correlation observed across environments suggest that similar selection indices can be constructed to identify ideotypes for the respective environments provided that the materials at Sheno are to be evaluated under similar circumstances (camber bed).

Table 6.10. Genotypic (G) and phenotypic (P) correlation coefficients of agronomic characters of barley lines evaluated at Keyit, Ethiopia, 2001.

Character	r type	DMA	PLH	SPL	NS/SP	GY/SP	NP/M2	GY/Plot	BM	HI	TKW
DHE	P	0.892***	-0.132	0.434***	0.224	0.395**	-0.143	0.197	0.333**	-0.209	0.403*
	G	0.924***	-0.169	0.459***	0.240	0.427***	-0.173	0.252*	0.451***	-0.269*	0.425*
DMA	P		-0.241	0.408***	0.264*	0.426***	-0.174	0.190	0.269*	-0.119	0.417*
	G		-0.290*	0.430***	0.281*	0.457***	-0.209	0.242	0.363**	-0.119	0.438*
PLH	P				0.049	0.201	0.078	0.284*	0.407***	-0.088	0.251*
	G				0.060	0.252*	0.109	0.421***	0.640***	-0.132	0.308*
SPL	P				0.069	0.314*	-0.001	0.497***	0.500***	0.197	0.547*
	G				0.076	0.344**	-0.001	0.645***	0.689***	0.257*	0.059
NS/SP	P					0.821***	-0.581***	0.074	-0.125	0.344**	-0.115
	G					0.909***	-0.720***	0.098	-0.174	0.457***	-0.125
GY/SP	P						-0.624***	0.188	0.019	0.291*	0.405*
	G						-0.778***	0.249*	0.027	0.382**	0.441*
NP/M²	P							0.419***	0.563***	-0.099	-0.214
	G							0.618***	0.882***	-0.147	-0.261
GY/plot	P								0.867***	0.459***	0.187
	G								0.021	0.725***	0.241
BM	P									0.016	0.243
	G									0.027	0.333*
HI	P										-0.109
	G										-0.141

Table 6.11. Genotypic (G) and phenotypic (P) correlation coefficients of agronomic characters of barley lines evaluated at Sheno, Ethiopia, 2001

Character	r type	DMA	PLH	SPL	NS/SP	GY/SP	NP/M2	GY/Plot	TKW
DHE	P	0.852***	0.272*	0.401***	0.309*	0.684***	-0.341***	0.377**	0.660***
	G	0.881***	0.528***	0.417***	0.341**	0.784***	-0.386**	0.471***	0.728***
DMA	P		0.151	0.484***	0.382*	0.773***	-0.382**	0.372**	0.709***
	G		0.278*	0.514***	0.425***	0.891***	-0.447***	0.468***	0.781***
PLH	P			0.153	0.0.136	0.277*	0.144	0.364**	0.202
	G			0.308*	0.246*	0.568***	0.134	0.556***	0.397*
SPL	P				0.234	0.467***	-0.159	0.326**	0.435***
	G				0.209	0.499***	-0.194	0.387**	0.497***
NS/SP	P					0.702***	-0.388**	0.223	0.122
	G					0.788***	-0.538***	0.229	0.154
GY/SP	P						-0.446***	0.421***	0.647***
	G						-0.623***	0.529***	0.791***
NH/M²	P							0.476***	0.423***
	G							0.504***	0.503***
GY/plot	P								0.299*
	G								0.399**

* P = 0.05; ** P = 0.01; *** P = 0.001; r = correlation ; DHE = days to heading; DMA = days to maturity; PLH = plant height; SPL = spike length; NS/SP = number of seeds spike^{-1}; GY/SP = grain yield spike^{-1}; NH/M2 = number of plants per square meter; GY/plot = grain yield plot^{-1}; TKW = 1000-kernel weight.

Table 6.12. Genotypic (G) and phenotypic (P) correlation coefficients of agronomic characters of barley lines from combined data at Sheno and Keyit, Ethiopia, 2001.

Character	r type	PLH	SPL	NS/SP	GY/SP	NH/M2	GY/Plot	TKW
DMA	P	-0.037	0.518***	0.337**	0.685***	-0.341**	0.313*	0.737***
	G	-0.049	0.656***	0.462***	0.961***	-0.532***	0.532***	0.905***
PLH	P		0.111	0.059	0.110	0.091	0.267*	0.139
	G		0.159	0.064	0.177	0.075	0.339**	0.279*
SPL	P			0.145	0.428***	-0.145	0.474***	0.199
	G			0.129	0.509***	-0.224	0.677***	0.883***
NS/SP	P				0.733***	-0.481***	0.154	0.050
	G				0.961***	-0.820***	0.154	0.092
GY/SP	P					-0.581***	0.145	0.561***
	G					-0.987***	0.178	0.862***
NH/M^2	P						0.323*	-0.415***
	G						0.471***	-0.696***
GY/Plot	P							0.154
	G							0.316*

* P = 0.05; ** P = 0.01; *** P = 0.001

r = correlation

6.4.6. Direct and indirect association of characters

Among the nine characters number of heads per square meter had the highest direct effect (0.792) followed by grain filling period and days to heading, the latter two exerting comparable direct effects (0.369 and 0.368, respectively) on grain yield. Similarly, the direct effects of number of seeds per spike and grain yield per spike on grain yield are comparable (Table 6.13). All characters but days to maturity and plant height had positive direct effects on grain yield. The indirect effects are generally very low compared to the direct effects. Opposite to this is days to maturity in which its indirect effect via grain filling period (0.268) is higher than its direct effect on grain yield suggesting the influence of grain filling period on grain yield. Higher number of heads per square meter resulted in a fewer number of seeds per spike and grain yield per spike, but contributed much to final grain weight, however (Figure 6.6). This relationship was also reflected in the simple correlation coefficients confirming that this character can be used as indirect selection criteria for grain yield. In the simple correlation analysis, days to maturity and plant height had significant positive association with grain yield (Table 6.13). Path analysis revealed, however, that both of these characters had a negative direct influence on grain production.

It has been reported that grain yield in barley depended largely on spikes per square meter followed by kernels per spike (Garcia del Moral et al., 1991; Dofing, 1997;

Sinebo, 2002). Proliferation of tillers is one of the first developmental processes in small grains and for this reason the spike number per square meter may exercise direct influence on all other characters (Garcia del Moral et al., 1991). Other workers (Mandal & Dana, 1993; El-Hennawy, 1997; Naik et al., 1998) noted that grains per spike is an important direct contributor to grain yield whereas length of main ear and weight of grains per ear are indirect contributors to grain yield (Verma et al., 1998). Rasmusson & Cannel (1970) discussed the argument against selection for a maximum genetic potential for number of heads per square meter in that higher number of heads per unit area and kernels per head could result in a large early demand on nutrients and water which could then become limiting at later, more critical stages of growth. Selection for higher genetic potential for kernel weight would appear advantageous in all environments since kernel weight is the last component to be developed in the ontogeny of the plants and does not result in a compensating change in the other components. Against the view of Rasmusson and Cannel (1970), the dependence of grain yield on number of heads per square meter and grain yield per spike appears to be an advantage for environments like north Shewa where there is no moisture limitation at grain filling period. However, in some years, early cessation of rain in September with subsequent desiccating wind and frost may limit barley yield in this particular environment and makes concomitant selection for number of heads per square meter and grain yield per spike, inevitable. The strong negative association between grain yield per spike and number of heads per square meter and the negative direct effect of number of heads per square meter on number of seeds per spike and grain yield per spike makes simultaneous selection difficult, however, and is in fact a challenge to the breeder.

A longer vegetative period (emergence to heading) had a positive association with grain yield and its positive direct effect appears necessary to develop a sufficient number of leaves and provide photo-assimilate during grain filling period. However, the vegetative period should not be so long that it delays maturity beyond the limit and subject the crop to terminal stress (frost and dry desiccating wind). Delayed maturity had a negative direct effect on grain yield. The negative effects of the vegetative period on grain yield in low nitrogen and terminal moisture stress environments reported by Sinebo (2002) need consideration because its positive effect discussed in this experiment is under no nitrogen limitation.

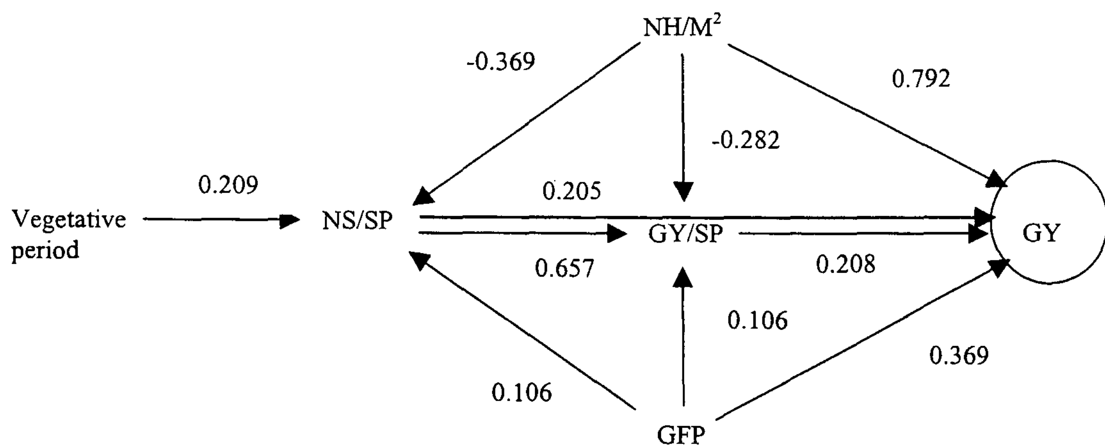

Figure 6.6. Path diagram illustrating the direct effects of one character on the other and on final grain yield of barley landrace lines evaluated at Keyit and Sheno, 2001.

Table 6.13. Path coefficients showing direct and indirect effects of agronomic characters on grain yield in barley landrace lines evaluated at Keyit and Sheno, 2001.

Characters	**Direct effect**	Indirect effects via									Correlation With GY
		DHE	DMA	PLH	SPL	NS/SP	GY/SP	NH/M^2	TKW	GFP	
DHE	**0.368**	-	-0.067	-0.035	0.061	0.036	0.081	-0.044	0.077	-0.153	0.327***
DMA	**-0.203**	0.121	-	0.016	0.103	0.052	0.123	-0.234	0.067	0.268	0.314**
PLH	**-0.121**	0.108	0.027	-	0.035	0.038	0.054	0.076	0.057	-0.127	0.247*
SPL	**0.252**	0.089	-0.082	-0.017	-	0.045	0.091	-0.069	0.090	0.079	0.479***
NS/SP	**0.205**	0.068	-0.052	-0.022	0.056	-	0.164	-0.322	0.000	0.041	0.138NS
GY/SP	**0.208**	0.144	-0.119	-0.031	0.109	0.162	-	-0.463	0.089	0.105	0.203NS
NH/M^2	**0.792**	-0.021	0.059	-0.012	-0.022	-0.084	-0.122	-	-0.049	-0.090	0.453***
TKW	**0.185**	0.153	0.074	-0.037	0.123	0.000	0.099	-0.209	-	0.017	0.258*
GFP	**0.369**	-0.152	-0.147	0.014	0.055	0.023	0.059	-0.193	0.009	-	0.064NS

Abbreviations are as described in table 5.11. *, **, and *** are significant at $P \leq 0.05$, $P \leq 0.01$ and $P<0.001$, respectively.

Multiple regression was applied to the data and an acceptable level of multicolinearity was detected among the yield components when considered all together. This was seen from the variance inflation factor (VIF), a measure of multicolineartity, which was less than 10 for each variable. The inclusion of grain filling period introduced lack of fit and error to the regression model, however, and

was excluded from the analysis. Metzeger et al. (1984) also reported lack of association between grain filling period and grain yield although path analysis revealed that this character has direct positive effect. The analysis revealed highly significant regression coefficients (b values) for number of heads per square meter across environments and in combined data (Table 6.14). Similarly in a stepwise regression analysis, the highest and significant R-squared increment was detected for number of heads per square meter followed by grain yield per spike (Table 6.14) substantiating the importance of these two characters in determining grain yield.

Table 6.14.Multiple and stepwise regression analyses on yield and yield components of landrace lines evaluated at Keyit and Sheno, Ethiopia in 2001.

Variable	Sheno		Keyit		Sheno & Keyit	
	R.coefficient	Prob.	R.coefficient	Prob.	R.coefficient	Prob.
Intercept	-503.936	0.003	-784.495	0.000	678.070	0.017
Days to heading	1.002	0.601	1.385	0.125	1.090	0.426
Days to maturity	0.803	0.600	2.144	0.033	2.796	0.058
Spike length	1.434	0.715	-0.992	0.816	15.332	0.011
No. of seeds spike^{-1}	1.608	0.184	1.277	0.303	2.278	0.228
Grain yield spike^{-1}	40.960	0.119	7.720	0.796	4.808	0.917
No. heads m^{-2}	0.552	0.000	0.442	0.000	0.732	0.000
1000-kernel wt.	3.251	0.067	2.895	0.065	2.794	0.243
R^2	0.72		0.85		0.65	

Stepwise regression

Variable	Sheno		Keyit		Sheno & Keyit	
	R^2 increment	Prob.	R^2 increment	Prob.	R^2 increment	Prob.
Days to heading	0.0061	0.271	0.0099	0.057	0.0097	0.218
Days to maturity	0.0058	0.282	0.0164	0.015	0.0182	0.090
Spike length	0.0011	0.633	0.0003	0.702	0.0784	0.000
No. of seeds spike^{-1}	0.0056	0.292	0.0002	0.780	0.0017	0.600
Grain yield spike^{-1}	0.2478	0.000	0.0199	0.007	0.1405	0.000
No. heads m^{-2}	0.5491	0.000	0.0757	0.000	0.3878	0.000
1000-kernel wt.	0.2092	0.030	0.0062	0.123	0.0054	0.360
R^2	0.70		0.84		0.61	

R-coefficient=regression coefficient (b values)

6.4.7. The biochemical basis of variation within and between landraces

SDS-PAGE of seed storage proteins was carried out to investigate the extent of genetic differences within and among the landraces evaluated under field conditions to confirm the information obtained from quantitative morphological data. The genetic relationship among the different lines is presented in Figure 6.7. Of the 1953 pair-wise comparisons involving the pure lines and the standard checks (HB-42 and Misrach), 29 (i.e.1.5 %) were genetically identical. Eighteen percent of the overall pair-wise comparisons had a genetic distance $\geq$ 0.700.The overall genetic distance among lines for the entire landraces ranged from 0.000 to 0.943 with a mean of 0.574$\pm$0.13 while distance from pair-wise comparisons of lines within most of the landraces did not exceed 0.666 except between some of the lines within Kessele that revealed dissimilarity as high as 0.781 (Table 6.15). Moreover, within landraces mean genetic distance was very low ranging from 0.094 to 0.444 except for Kessele and Key Ferke that had a mean genetic distance of 0.587 and 0.519, respectively. Lines from Mage and Nech Gebs had shown genetic similarity (i.e. genetic distance equals to zero) in 60 % of the pair-wise comparisons indicating their relative genetic uniformity compared to the others. This comparison of variation within and among landraces displayed more variation among landraces than within landraces which supported data from quantitative morphological characters.

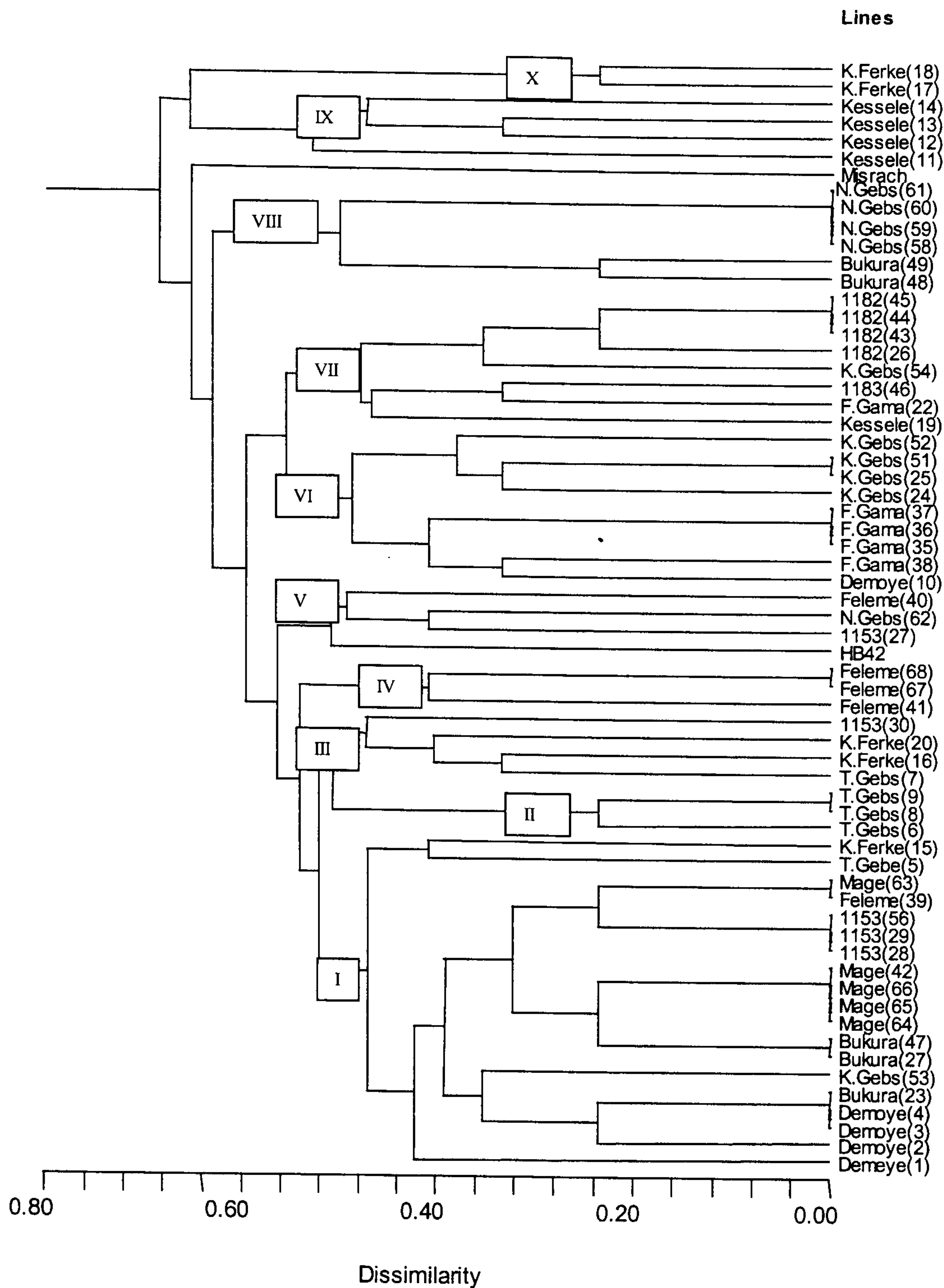

Figure 6.7. Dendrogram illustrating genetic relationships among barley landrace lines based on SDS-PAGE of seed storage protein

A comparison of agronomic performance among genetically identical lines was done based on data from Keyit where soil and related variables were supposed to be less, uniform across the experimental field and landraces expressed their genetic potential to a maximum. Differences for agronomic characters between the genetically identical lines were generally negligible except in few instances where as much as seven days difference in days to heading and 11 days in days to maturity was observed between Feleme(67) and Feleme(68). Among the characters, differences for grain yield were relatively high denoting how variations due to non genetic factors could be so high when evaluating materials for grain yield (Table 6.16). These differences among the paired comparisons were not statistically significant except between Nech Gebs(60) and Nech Gebs(61). Hence, differences for agronomic characters between genetically identical lines are purely environmental. Similar comparison was done between genetically distant lines. In this group, most of the lines compared showed significant differences for different agronomic characters (Table 6.17). Feres Gama(36) vs Nech Gebs(59), Feres Gama(36) vs Nech Gebs(61) and Feres Gama(36) vs Nech Gebs(62) are the exceptions.

From these results, it is possible to understand that it requires careful evaluation and selection from very large samples within some of the farmers' cultivars to make slight improvement for agronomic characters. Apparent progress through selection for yield and yield components within most of the farmers' cultivars from this particular environment is unlikely, however. Farmers often do not clean their seeds and are unable to maintain "true to type" different cultivars. Mixing of seeds of different cultivars is possible on a threshing ground not cleaned carefully, and therefore mixtures are planted. Rouging after heading is also difficult especially if the cultivars have the same seed colour and phenology. Therefore, it is possible that all these factors can lead to variability within landraces without neglecting the existence of biotypes within farmers' cultivars. Overall, the assessments of genetic variability of the farmers' cultivars both from morphological and protein profiles perspectives illustrated less variability within farmers cultivars. Hence, progress through pure line selection cannot be warranted. Instead, it can be suggested that carefully rouging the inferior biotypes or off types from farmers' cultivars may help exploit their maximum genetic potential rather than attempting to improve their productivity through pure line selection and evaluation methods.

Table 6.15. Genetic distances among lines within each landrace or accession (evaluated at Keyit and Sheno) based on SDS-PAGE of seed storage proteins.

Landrace or accessions and lines (numbered serially) within each landrace or accession											
Acc.1153		GD*	Demoye		GD	Key Ferke		GD	Nech Gebs		GD
27[a]	28[b]	0.527	1	2	0.408	15	16	0.408	58	59	0.000
27	29	0.527	1	3	0.471	15	17	0.623	58	60	0.000
27	30	0.577	1	4	0.471	15	18	0.577	58	61	0.000
27	56	0.527	1	10	0.577	15	20	0.527	58	62	0.623
28	29	0.000	2	3	0.235	16	17	0.666	59	60	0.000
28	30	0.408	2	4	0.235	16	18	0.623	59	61	0.000
28	56	0.000	2	10	0.408	16	20	0.333	59	62	0.623
29	30	0.408	3	4	0.000	17	16	0.235	60	61	0.000
29	56	0.000	3	10	0.471	17	20	0.577	60	62	0.623
30	56	0.408	4	10	0.471	18	20	0.623	61	62	0.623
Mean		**0.338**			**0.375**			**0.519**			**0.249**
Acc.1182		GD	Feres Gama		GD	Kessele		GD	Tikur Gebs		GD
26	43	0.235	22	35	0.408	11	12	0.527	5	6	0.408
26	44	0.235	22	36	0.408	11	13	0.527	5	7	0.471
26	45	0.235	22	37	0.408	11	14	0.527	5	8	0.471
26	146	0.471	22	38	0.577	11	19	0.745	5	9	0.471
43	44	0.000	35	36	0.000	12	13	0.333	6	7	0.408
43	45	0.00	35	37	0.000	12	14	0.471	6	8	0.235
43	46	0.527	35	38	0.408	12	19	0.781	6	9	0.235
44	45	0.000	36	37	0.000	13	14	0.471	7	8	0.471
44	46	0.527	36	38	0.408	13	19	0.781	7	9	0.471
45	46	0.527	37	38	0.408	14	19	0.707	8	9	0.000
Mean		**0.275**			**0.302**			**0.587**			**0.364**
Key Gebs		GD	Feleme		GD	Mage		GD	Bukura		GD
24	25	0.333	39	40	0.471	42	63	0.235	23	47	0.471
24	51	0.333	39	41	0.471	42	64	0.000	23	48	0.471
24	52	0.471	39	67	0.527	42	65	0.000	23	49	0.623
24	53	0.577	39	68	0.527	42	66	0.000	23	50	0.577
24	54	0.408	40	41	0.577	63	64	0.235	47	48	0.000
25	51	0.000	40	67	0.527	63	65	0.235	47	49	0.408
25	52	0.333	40	68	0.527	63	66	0.235	47	50	0.333
25	53	0.577	41	67	0.408	64	65	0.000	48	49	0.408
25	54	0.527	41	68	0.408	64	66	0.000	48	50	0.333
51	52	0.333	67	68	0.000	65	66	0.000	49	50	0.235
51	53	0.577	**Mean**		**0.444**			**0.094**			**0.386**
51	54	0.527									
52	53	0.577									
52	54	0.623									
53	54	0.408									
Mean		**0.440**									

* genetic distance; a & b refer to serial identification numbers given to each line within a landrace (eg. 27 represents 1153(27).

Table 6.16. Comparative values and differences (absolute values) in agronomic characters of genetically identical lines (significantly different values are indicated in bold).

Lines compared	DMA		D	DHE		D	GY		D	GY/SP		D	NS/SP		D	PLH		D	SPL		D
1153(28) vs 1153(29)	124	124	0	81	81	0	577.8	603.4	25.6	4.07	4.00	0.07	72	72	0	104	102	2	7.8	7.9	0.1
1153(28) vs 1153(56)	124	124	0	81	81	0	577.8	573.6	4.2	4.07	3.57	**0.50**	72	69	3	104	106	2	7.8	7.5	0.3
1153(29) vs 1153(29)	124	124	0	81	81	0	603.4	573.6	29.8	4.00	3.75	0.25	72	69	3	102	106	4	7.9	7.5	0.4
1182((43) vs 1182(44)	123	121	2	80	78	2	459.1	337.5	121.6	2.50	2.42	0.08	55	55	0	105	97	8	4.4	4.6	0.2
1182(43) vs 1182(45)	123	124	1	80	81	1	459.1	404.8	54.3	2.50	2.52	0.02	55	50	5	105	96	9	4.4	4.8	0.4
1182(44) vs1182(45)	121	124	3	78	81	**3**	337.5	404.8	67.3	2.42	2.45	0.03	55	50	5	97	96	1	4.6	4.8	0.2
Bukura(23) vs Demoye(3)	130	126	**4**	80	78	2	547.2	665.9	118.7	3.47	2.97	**0.50**	62	62	0	102	102	0	5.3	8.1	**2.8**
Bukura(23) vs Demoye(4)	130	125	**5**	80	78	2	547.2	590.6	43.4	3.47	3.05	0.42	62	60	2	102	95	7	5.3	7.8	**2.5**
Bukura(47) vs Bukura(48)	129	121	**8**	79	75	**4**	580.7	613.6	32.9	3.35	2.22	**1.13**	63	52	**11**	104	91	13	5.7	5.8	0.1
Demoye(3) vs Demoye(4)	126	125	1	78	78	0	665.9	590.6	75.3	2.97	3.05	0.08	62	60	2	102	95	7	8.1	7.8	0.3
F.Gama(35) vs F.Gam(36)	141	139	2	86	92	**6**	671.2	719.2	48.0	3.07	3.55	**0.48**	56	62	6	92	95	3	7.8	8.7	**0.9**
F.Gama(35) vs F.Gam(37)	141	142	1	86	87	1	671.2	745.9	74.7	3.07	3.00	0.07	56	53	3	92	98	6	7.8	7.8	0.0
F.Gama(36) vs F.Gam(37)	139	142	3	92	87	**5**	719.1	745.9	26.8	3.55	3.00	0.55	62	53	**9**	95	98	3	8.7	7.8	**0.9**
Feleme(39) vs Mage(63)	123	123	0	79	77	2	687.7	687.8	0.1	2.30	2.37	0.07	52	52	0	104	97	7	7.6	6.4	**1.2**
Feleme(67) vs Feleme(68)	135	124	**11**	87	80	**7**	684.8	608.5	76.3	2.75	2.25	0.50	54	54	0	103	100	3	7.2	6.0	**1.2**
K.Gebs(25) vs K.Gebs(51)	122	122	0	80	79	1	614.5	630.6	16.1	2.40	2.30	0.10	59	50	**9**	95	90	5	6.3	6.2	0.1
Mage(42) vsMage(64)	122	123	1	79	79	0	573.4	721	147.6	2.22	2.20	0.02	54	51	3	93	97	4	6.0	6.2	0.2
Mage(42) vs Mage(65)	122	119	3	79	75	**4**	573.4	592.3	18.9	2.22	2.17	0.05	54	55	1	93	94	1	6.0	6.1	0.1
Mage(42) vs Mage(66)	122	123	1	79	79	0	573.4	610.2	36.8	2.22	2.22	0.00	54	50	4	93	97	4	6.2	6.0	0.0
Mage(64) vs Mage(65)	123	119	4	79	75	**4**	721	592.3	128.7	2.20	2.17	0.03	51	55	4	97	94	3	6.2	6.1	0.1
Mage(64) vs Mage(66)	123	123	0	79	79	0	721	610.2	110.8	2.20	2.22	0.02	51	50	1	97	97	0	6.2	6.0	0.2
Mage(65) vs Mage(66)	119	121	2	75	79	**4**	592.3	610.2	17.9	2.17	2.22	0.05	55	50	5	94	97	3	6.1	6.0	0.1
N.Gebs(58) vs N.Geb(59)	144	140	**4**	89	91	2	638.2	636.1	2.1	3.50	3.57	0.07	67	68	1	95	95	0	8.2	8.9	0.7
N.Gebs(58) vs N.Geb(60)	144	143	1	89	91	2	638.2	547.1	91.1	3.50	3.62	0.12	67	71	4	95	94	1	8.2	8.8	0.6
N.Gebs(58) vs N.Geb(61)	144	138	**6**	89	90	1	638.2	761.3	123.1	3.50	3.42	0.08	67	68	1	95	99	4	8.2	8.5	0.3
N.Gebs(59) vs N.Geb(60)	140	143	3	91	91	0	636.1	547.1	89.0	3.57	3.62	0.05	68	71	3	95	94	1	8.9	8.8	0.1
N.Gebs(59) vs N.Geb(61)	140	138	2	91	90	1	636.1	761.3	125.2	3.57	3.42	0.15	68	68	0	95	99	4	8.9	8.5	0.4
N.Geb(60) vs N.Geb(61)	143	138	5	91	90	1	547.1	761.3	**214.2**	3.62	3.42	0.20	71	68	3	94	99	5	8.8	8.5	0.3
T.Gebs(8) vs T.Gebs(9)	124	125	1	81	81	0	710.1	811.7	101.6	2.92	3.12	0.20	54	51	3	105	109	4	8.0	8.6	0.6
LSD (P=0.05)	3.14			2.49			174.14			0.45			7.43			7.89			0.88		

Table 6.17. Comparative values and differences (absolute values) in agronomic characters of genetically distant lines (significantly different values indicated in bold).

Lines compared	GD	DHE		D	DMA		D	GY		D	GY/SP		D	SPL		D	NS/SP		D
1153(29) vs Bukura(49)	0.816	81	80	**1**	124	129	**5**	603.4	618.3	14.9	4.00	3.42	**0.58**	7.8	4.8	**3.0**	72	67	5
1153(30) vs Bukura(49)	0.816	81	80	**1**	123	129	**6**	554.7	618.3	63.6	3.40	3.42	0.02	7.5	4.8	**2.7**	68	67	**1**
Bukura(47) vs Kessele(11)	0.850	79	80	**1**	129	124	**5**	580.7	764.8	**184.1**	3.35	2.80	**0.55**	5.7	7.2	**1.5**	63	58	5
Bukura(48) vs F.Gama(35)	0.816	75	86	**11**	121	141	**20**	613.6	671.2	57.6	2.22	3.07	**0.85**	5.8	7.7	**1.9**	52	56	4
Bukura(48) vs K.Ferke(16)	0.816	75	77	2	121	124	3	613.6	822.1	**208.5**	2.22	2.95	**0.73**	5.8	6.5	0.7	52	61	**9**
Bukura(48) vs K.Gebs(51)	0.816	75	79	**4**	121	122	1	613.6	630.6	17.0	2.22	2.30	0.08	5.8	6.2	0.4	52	50	2
Bukura(48) vs K.Gebs(52)	0.850	75	79	**4**	121	123	2	613.6	556.8	56.8	2.22	1.81	**0.41**	5.8	6.9	**1.1**	52	28	**24**
Bukura(48) vs K.Gebs(53)	0.850	75	79	**4**	121	123	2	613.6	647.9	34.3	2.22	2.30	0.08	5.8	6.6	0.8	52	56	4
Bukura(48) vs K.Gebs(54)	0.850	75	79	**4**	121	123	2	613.6	598.8	14.8	2.22	2.25	0.03	5.8	7.0	**1.2**	52	47	5
Bukuar(48) vs Kesele(11)	0.882	75	80	**5**	121	124	3	613.6	764.8	151.2	2.22	2.80	**0.58**	5.8	7.2	**1.4**	52	58	6
Bukura(49) vs F.Gama(35)	0.882	80	86	**6**	129	141	**12**	618.3	671.2	52.9	3.42	3.07	0.35	4.8	7.7	**2.9**	67	56	**11**
Bukura(49) vs Feleme(40)	0.816	80	88	**8**	129	135	**6**	618.3	692	73.7	3.42	2.22	1.20	4.8	8.0	**3.2**	67	54	**13**
Bukura(49) vs K.Ferke(16)	0.816	80	77	**3**	129	124	**5**	618.3	822.1	**203.8**	3.42	2.95	**0.47**	4.8	6.5	**1.7**	67	61	6
Bukura(49) vs K.Ferke(20)	0.816	80	78	2	129	125	**4**	618.3	643.8	25.5	3.42	2.77	**0.65**	4.8	6.8	**2.0**	67	58	**9**
Bukura(49) vs K.Gebs(51)	0.882	80	79	1	129	122	**7**	618.3	630.6	12.3	3.42	2.30	**1.12**	4.8	6.2	**1.4**	67	50	**17**
Bukura(49) vs K.Gebs(52)	0.850	80	79	1	129	123	**6**	618.3	556.8	61.5	3.42	1.81	**1.61**	4.8	6.9	**2.1**	67	28	**39**
Bkura(49) vs K.Gebs(53)	0.850	80	79	1	129	123	**6**	618.3	647.9	29.6	3.42	2.30	**1.12**	4.8	6.6	**1.8**	67	56	**11**
Bukura(49) vs K.Gebs(54)	0.850	80	79	1	129	123	**6**	618.3	598.8	19.5	3.42	2.25	**1.17**	4.8	7.0	**2.2**	67	47	**20**
Bukura(49) vs Kessele(11)	0.943	80	80	0	129	124	**5**	618.3	764.8	146.5	3.42	2.80	**0.62**	4.8	7.2	**2.4**	67	58	**9**
Bukura(50) vs F.gama(35)	0.816	80	86	**6**	126	141	**15**	599.7	671.2	71.5	3.35	3.07	0.28	5.1	7.7	**2.6**	63	56	7
Bukura(50) vs Kessele(11)	0.882	80	80	0	126	124	2	599.7	764.8	165.1	3.35	2.80	**0.55**	5.1	7.2	**2.1**	63	58	5
Demoye(1) vs Demoye(10)	0.816	77	77	0	125	123	2	558.7	552.7	6.0	3.10	2.20	**0.90**	8.1	5.5	**2.6**	62	46	**16**
Demoye(4) vs Mage(65)	0.816	78	75	**3**	125	119	**6**	590.6	592.3	1.7	3.05	2.17	**0.88**	7.7	6.0	**1.7**	60	55	5
Demoye(10) vs Feleme(68)	0.816	77	80	**3**	123	123	0	552.7	608.5	55.8	2.20	2.25	0.05	5.5	6.0	0.5	46	55	**9**
Kessele(11) vs N.Gebs(59)	0.816	80	91	**11**	124	140	**16**	764.8	636.1	128.7	2.80	3.57	**0.77**	7.2	8.9	**1.7**	58	68	**10**
Kessele(11) vs N.Gebs(60)	0.816	80	91	**11**	124	143	**19**	764.8	547.1	**217.7**	2.80	3.62	**0.82**	7.2	8.8	**1.6**	58	71	**13**
Kessele(11) vs N.Gebs(61)	0.816	80	90	**10**	124	138	**14**	764.8	761.3	3.5	2.80	3.42	**0.62**	7.2	8.5	**1.3**	58	68	**10**
Kessele(11) vs N.Gebs(62)	0.816	80	93	**13**	124	141	**17**	764.8	642.9	121.9	2.80	3.45	**0.65**	7.2	8.5	**1.3**	58	64	6
F.Gama(36) vs N.Gebs(59)	0.782	92	91	1	139	140	1	719.1	636.1	83.0	3.55	3.57	0.02	8.6	8.9	0.3	62	68	6
F.Gama(36) vs N.Gebs(60)	0.782	92	91	1	139	143	**4**	719.1	547.1	172.0	3.55	3.62	0.07	8.6	8.8	0.2	62	71	**9**
F.Gama(36) vs N.Gebs(61)	0.782	92	90	2	139	138	1	719.1	761.3	42.2	3.55	3.42	0.13	8.6	8.5	0.1	62	68	6
F.Gama(36) vs N.Gebs(62)	0.782	92	93	1	139	141	2	719.1	642.9	76.2	3.55	3.45	0.10	8.6	8.5	0.1	62	64	2
K.Gebs(52) vs Kessele(19)	0.782	79	80	1	123	123	0	556.8	674.3	117.5	1.81	2.81	**1.00**	6.9	8.1	**1.2**	28	49	**21**
K.Gebs(53) vs Kessele(19)	0.782	79	80	1	123	123	0	556.2	674.3	118.1	2.30	2.81	**0.51**	6.6	8.1	**1.5**	56	49	7
Mean		80	83	2.59	127	129	2.79	633.8	659.8	26.1	2.99	2.82	0.17	6.26	7.23	0.96	59.6	56.6	3
LSD(P=0.05)		2.49			3.14			174.1			0.44			0.88			7.42		

GD refers to genetic distance. D represents difference in agronomic characters among the lines compared.

Cluster analysis based on quantitative characters (standardized values) and data from SDS-PAGE of seed storage proteins provided fairly similar grouping of landrace lines in that lines derived from the same landrace were clustered together (Figures 6.4 & 6.7). Correlation between genetic distance values resulting from the two measures of genetic relationship indicated a highly significant association although the value is small (r=0.199). The morphological distance matrix formed based on Johns et al. (1997) also showed highly significant association (r=0.164) with genetic distance from SDS-PAGE and r=0.999 with that resulting from cluster analysis based on quantitative variables (standardized values). It can be discerned that the lack of fit between clusters resulting from morphological and SDS-PAGE data discussed in chapter IV could be because of the inclusion of qualitative characters with major phenotypic effects that influenced the pattern of clustering. Hence, it would be suggested to focus on quantitative characters and see relationships among breeding materials since they are traits of interest to the breeder also. Moreover, in the case of barley, yield is a priority to farmers and their selection criteria are also for quantitative rather than qualitative characters in most cases.

6.5. Conclusions

It is a well-known fact that selection can act effectively only on heritable characteristics and that selection cannot create variability. Variability is, therefore, a key factor that determines the amount of progress expected from selection. Genetic variability and response to selection of farmers' barley cultivars from north Shewa was assessed based on data from field experiments at Keyit and Sheno. Emphasis was given to farmers' cultivars because of increasing interest and concern towards their utilization in a breeding program. The result demonstrated that variability was higher for reproductive characters (spike length, number of seeds per spike and grain yield per spike) than for days to heading and maturity. Estimates of genetic advance and cluster analysis based on data of quantitative characters illustrated lesser variability within than between farmers' cultivars that was further verified by SDS-PAGE of seed storage proteins. One may argue that the sample size per farmers' cultivars was too small to conclude that selection within farmers' cultivars is less effective. However, it was observed that single plants taken from each cultivar and planted for seed increase were very similar and

visually indistinguishable. SDS-PAGE data from 15 samples per cultivar (discussed in chapter IV) also showed less variation within cultivars which supported the decision to reduce sample size for field evaluation. Hence, it can be concluded that, genetic gain through selection for yield and yield components within the farmers' cultivars from this particular environment is expected to be minimal. Carefully excluding the inferior types to upgrade their yield potential is suggested rather than attempting to improve them through extensive pure line selection and evaluation methods. Evaluation of landraces from other sources from within or outside the environment is suggested to get better yielding landraces than the existing ones. In the long term, crossing may be scheduled between cultivars, which based on morphological characters and protein profiles, appear to be diverse in order to maximize the potential gain from selection in the progeny.

An investigation of the relationship between yield and yield components revealed that yield is primarily positively influenced by number of heads per square meter. Number of seeds per spike, grain yield per spike, vegetative period and grain filling duration had also positive direct effects on grain yield. A given cultivar needs to maintain a balance between these characters in order to give an acceptable level of yield to the farmers in this environment. However, the negative direct effects of number of heads per square meter on number of seeds per spike and grain yield per spike makes simultaneous selection for these characters difficult.

CHAPTER VII

VARIABILITY AMONG BARLEY LANDRACES IN RESPONSE TO WATERLOGGING STRESS: GROWTH AND NUTRIENT UPTAE

7.1. Abstract

The central highlands occupy a significant portion of vertisols in Ethiopia and barley production in some parts of this region is constrained by seasonal waterlogging. The practice of soil burning (*guie*) to facilitate drainage and selecting tolerant landraces such as Mage is a well recognized strategy of farmers designed to cope with the effects of waterlogging. The aim of this study was to investigate differences in growth, nutrient uptake and concentration under waterlogging stress between Mage(07) which is a pure line from a landrace claimed to be tolerant to waterlogging stress, Feres Gama(37), a pure line from the susceptible landrace to waterlogging, and Feleme(68), a pure line from a landrace well known in the area. A three week waterlogging treatment was done in the greenhouse and was compared with a free drainage treatment. Waterlogging reduced the total number of tillers per pot of the tolerant landrace Mage(07) and Feleme(68) by 43 % and 48 %, respectively compared to the control plants while it was only 23 % for the susceptible landrace Feres Gama(37). Total shoot dry matter accumulation under waterlogged condition was comparable for all three landraces in spite of the largest number of tillers produced by Feres Gama(37). Tillers produced by Feres Gama(37) had significantly (P=0.05) less dry matter than Mage(07) and Feleme(68), however. Differences between the susceptible and tolerant landraces in response to waterlogging were largely due to less dry matter accumulation of the tillers and slower growth in the susceptible landrace compared to the tolerant landrace. Moreover, apparent differences were noticed in P concentration and uptake between the tolerant and susceptible landraces, the effect being less for the tolerant landrace Mage(07) than for the susceptible landrace Feres Gama(37). Difference in N concentration of shoots between Feres Gama(37) and Mage(07) was also observed although the magnitude was not comparable to that of P.

7.2. Introduction

Crop production in natural environments is subject to biotic and abiotic stresses although the degrees vary between environments. Waterlogging is one of the important environmental factors that influence plant composition and growth in many parts of the world (Blom et al., 1990). Waterlogging may be permanent for example in marshes, or may occur in a regular daily or seasonal pattern. The latter is commonly experienced in the highland vertisol areas of Ethiopia. The central highlands, including parts of north Shewa, are among the regions occupying a significant portion of vertisols in Ethiopia. The soils vary from dark and heavy black soils to red with high amount of clay particles that restrict drainage. Wheat, faba bean, chickpea and lentil are the predominant crops on black soils while barley is grown on the red or brown soils. Excessive water that occurs in the soil for short periods during the growing season as a result of intense rain in combination with restricted water flow is a major threat to crop production in such environments. Saturated conditions can adversely influence the growth of annual species such as barley within one week (Leyshon & Sheard, 1974) and the reduction will depend on the duration of waterlogging and the stage of development of the plant at the time of waterlogging.

The challenge of crop production on highland vertisol areas is partly overcome by traditional management practices such as hand made broad bed and furrows, ridge and furrow and opening furrows at regular intervals to drain the excess water from their fields. The latter, along with soil burning (guie) is common practice of farmers growing barley in north Shewa. Survey results indicated that about 30-40 % of the barley growers in Sheno to Chacha area in north Shewa plant barley using soil burning (Yirga et al., 1998). Moreover, selecting landraces among many others that fit to the system is a well recognized strategy of farmers to cope with the challenge they face. The landrace, Mage for example, is reported by farmers to be tolerant to waterlogging and is widely used (Yirga et al., 1998). Extensive studies have been done regarding soil burning and its effects on nutrients in the soil and crop performance (IAR, 1967; Sahlemedhin, 1987) but there is no experimental evidence to support genetic differences among landraces to waterlogging stress which is well appreciated by the farmers. Waterlogging tolerance in plants is achieved by one or more features that improve gas exchange, as well as various metabolic features that help maintain a sufficiency of energy production

to sustain cell integrity and avoid damage under waterlogging stress (Armstrong et al., 1994). Research elsewhere has demonstrated the existence of genotypic differences for tolerance to waterlogging in cereal crops (Davis & Hillman, 1988; Thomson et al., 1992; Ding & Musgrave, 1995; Huang et al., 1994a). One of the physiological effects of waterlogging is a decrease in uptake and transport of ions through roots (Drew & Sisiworo, 1979; Atwell & Steer, 1990). Differences in nutrient uptake and concentration between susceptible and tolerant genotypes have been reported (Huang et al., 1995). The purpose of this experiment was therefore to investigate differences in nutrient uptake and concentration among landraces supposed to differ in tolerance to waterlogging. Although the mechanism of waterlogging tolerance is very complex involving physiological, biochemical, anatomical and morphological changes or a combination of two or more of these mechanisms, the results from this study will provide information that can help as a starting point for further studies.

7.3. Materials and methods

7.3.1. Experimental materials

Two contrasting landraces differing in tolerance to waterlogging (Feres Gama, a susceptible landrace and Mage, a tolerant landrace) and Feleme, a known landrace in the area which is sometimes considered as better than Feres Gama in tolerance to waterlogging, were selected for this study based on survey information. Single plants from each landrace were selected to get a pure line. One pure line from each landrace was taken randomly for inclusion in the waterlogging experiment which are named hereafter as Mage(07), Feres Gama(37) and Feleme(68).

7.3.2. Experimental procedure

A red soil prepared for this experiment was sieved to avoid clods and packed in cylindrical asbestos pots of 75 cm deep and 20cm diameter. The experiment comprised of a combination of two waterlogging treatments (control and three weeks of waterlogging) and the three landrace lines providing a total of six treatments. Eight seeds of each treatment were planted per pot and thinned to five plants after germination. The treatments were arranged in a randomised complete block design with four replications. Holes at the bottom of the pots were sealed with silica gel to prevent

germination. The treatments were arranged in a randomised complete block design with four replications. Holes at the bottom of the pots were sealed with silica gel to prevent drainage for the waterlogging treatments and those for the control treatments were left open to facilitate drainage. Waterlogging treatment was started when seedlings reached nearly three to four leaf stage and the level of the water was maintained about 10 mm above the soil surface for the duration of the waterlogging period. The free drainage pots were watered daily to field capacity. In parts of north Shewa where soil burning (*guie*) is commonly practiced, rainfall starts late in June and ceases early in September. Barley planting is often done in dry soil and germinating seedlings reach three to four leaf stages in the second or third week of July depending on planting time. Since the soil remained dry for long, the soil is not so wet as to cause waterlogging and restrict growth and development of plants in the first two to three weeks in July. Therefore, three weeks of waterlogging at three to four leaf stage was considered sufficient to reflect the crop stage that is most likely to be subject to waterlogging stress under field conditions.

7.3.3. Data collection

Soil redox potential was measured both from the control and waterlogged treatments at the last day of the waterlogging period using a portable redox meter. Inserting the rod with a platinum tip nearly 10cm deep into the soil allowed the measurement of soil redox potential. Three weeks after the waterlogging treatment, the excess water in the waterlogged pots was drained and all plants from control and waterlogging treatments were harvested and separated into roots and shoots for nutrient analyses. Harvested shoots were rinsed with clean tap water to avoid possible contamination from foreign materials. The soil was gently washed off the roots and rinsed with tap water until it was clean from soil particles. Samples were oven dried at 80°C for 24 hours and dry matter was determined for roots and shoots. Shoot dry matter per plant and shoot dry matter per tiller were determined based on the total number of plants in the pot and number of tillers per plant, respectively. Plants were sampled from each treatment one week after waterlogging and rate of dry matter accumulation was determined as a difference in dry weight of samples from the last harvest and divided by the number of days elapsed between the two sampling periods.

7.3.4. Analyses of nutrients

Oven dried roots and shoots were ground to a fine powder with a coffee grinder. A 0.1g sample was weighed from each treatment for determination of nitrogen (N) concentration and 2g was taken for determination of Ca, Mg, Mn, Cu, Zn, Fe, Na, K, and P. N was determined using the Kjeldahl method. Samples for N determination were digested at 345°C with 95 % concentrated sulphuric acid (H_2SO4) in the presence of a catalyst. Steam distillation was carried out with NaOH and the distillate was titrated with 0.005 N H_2SO_4 until the colour changed from green to light pink. The amount of acid used was recorded after each titration. Calculation of N in the samples was done based on the difference of readings of the samples from the reading of the blank which is then multiplied by a factor 0.28. Samples for Ca, Mg, Mn, Cu, Zn, Fe, Na, K, and P determination were placed in porcelain crucibles for ashing in a muffle furnace at 540 °C for nearly three hours after which samples were dissolved by addition of 3ml of nitric acid (55 % HNO_3) and put in a muffle furnace again. Concentrations were then determined by an atomic absorption spectrometer (SpectrAA 300), except P with Spectrophotometer, after proper dilution. The total nutrient accumulation or uptake was calculated based on the concentrations and dry weight of the roots and shoots. Statistical analyses of data were carried out using Agrobase 2000 computer program.

7.4. Results and discussion

7.4.1. Changes in redox potential

Under anaerobic conditions a number of nutrient transformations are dependent on the intensity of reduction in the soil (Forster et al., 1993) and measures the tendency of the soil solution to receive or supply electrons (Meek, 1978). Therefore, in this study measuring soil redox potential was regarded as a step of primary importance since it provides useful information as to the degree of exhaustion of oxygen in the soil and also reflects soil chemical processes. At the end of three weeks of waterlogging period, soil redox potential was reduced significantly ($P < 0.001$) from an average of 387 mV in the free drainage pots to 129 mV in the waterlogged pots (Table 7.1). A rapid decrease of nitrate at 220mV, the appearance of soluble and reduced manganese at 200 mV, of soluble and reduced iron at 120 mV, and the disappearance of sulphate at –150 mV has been reported by Gambrell & Patrick (1978). The decline in the redox potential of the

Patrick (1978) and brought significant differences between free drainage and waterlogged plants in growth, nutrient concentration and uptake which shall be discussed later. The differences in redox potential within waterlogging treatments were so small and not significant reflecting the homogeneity of experimental pots and repeatability of measurements within treatments.

7.4.2. Growth differences

Few plants had begun tillering by the time waterlogging stress was imposed on the plants. Count of number of tillers per pot and per individual plants at the end of the waterlogging period revealed a detrimental effect of waterlogging on the tolerant landrace Mage(07) and Feleme(68). The total number of tillers and tillers per plant of the susceptible landrace Feres Gama(37) under waterlogged condition were significantly larger than the other two landraces (Table 7.1). The total number of tillers and tillers per plant of control plants did not differ between landraces. The reduction in total number of tillers of Mage(07) and Feleme(68) compared to the respective control plants was 43 % and 48 %, while similar comparison revealed only 23 % for Feres Gama(37). The expectation would have been smaller effects of waterlogging on Mage(07) a landrace claimed by farmers to be more tolerant to waterlogging than Feres Gama(37).

Although the landraces showed different responses in tillering under waterlogged conditions, no variation in the number of leaves was detected between landraces. Leaves from Feres Gama(37), especially of the smaller tillers, were narrow compared to those formed by Mage(07) and Feleme(68), however. Moreover, total shoot dry matter accumulation under waterlogged conditions was comparable for all three landraces in spite of the largest number of tillers formed by Feres Gama(37). This indicated that Feres Gama(37) achieved comparable shoot dry matter accumulation to Mage(07) and Feleme(68) at the expense of increased number of tillers whose dry matter yield is significantly ($P=0.05$) less than Mage(07) and Feleme(68). An increased number of tillers observed in the susceptible landrace Feres Gama(37) is, therefore, accompanied by a decrease in dry matter accumulation of individual tillers. It can be said that the effect of waterlogging on Feres Gama(37) is mainly on dry matter accumulation of the tillers and due to a decrease of shoot elongation. Although shoot elongation has not been measured in this experiment, the reduction of shoot growth can be envisaged from

Figure 7.1 in which visual contrasts between free drainage and waterlogged plants indicated a reduced top growth of Feres Gama(37) than Mage(07) and Feleme(68). Leyshon & Sheard (1974) also found that barley top growth is more sensitive to short-term waterlogging at an early growth stage, 14 days for example, than at 21, 28 or 35 days although younger plants are more capable of recovery than are the older plants. Reduced top growth and dry matter accumulation of tillers in the susceptible landrace Feres Gama(37) than Mage(07) and Feleme(68) can further be demonstrated by its relatively smaller rate of dry matter accumulation (mg/tiller/day) although the differences are not significant.

Shoot and root growth are mutually dependent and generally proceed concurrently in a linear fashion under optimum conditions. Hence, shoot : root ratio is expected to remain constant (De Wit, 1978). However, if one of the factors responsible for shoot and root growth becomes limiting, a shift in the shoot : root ratio may be expected. Despite this fact, in this experiment, a sharp decline of root dry matter accumulation was noticed in all three landraces following the three weeks of waterlogging but the shoot : root ratio remained unaffected. In agreement to this observation, Luxmoore et al. (1972) and Sojka et al. (1975) did not find an effect of reduced oxygen supply on the shoot : root ratio in wheat cultivars. A shift in shoot : root ratio depends on the distance from source to sink. In case of water shortage, for example, the growth of the root system is less affected than that of the shoot because the source of water is closest to the roots and the shoot to root ratio tends to decrease. Under waterlogged conditions, a lack of oxygen in the root medium reduces the extension growth of the root considerably and, as a result, the absorption rate is decreased while the absorbing surface per gram fresh weight of roots will increase by intensified branching of the roots and thus absorption may be improved (De Wit, 1978). As a result, the shoot growth may also increase and the shoot : root ratio may remain unaffected.

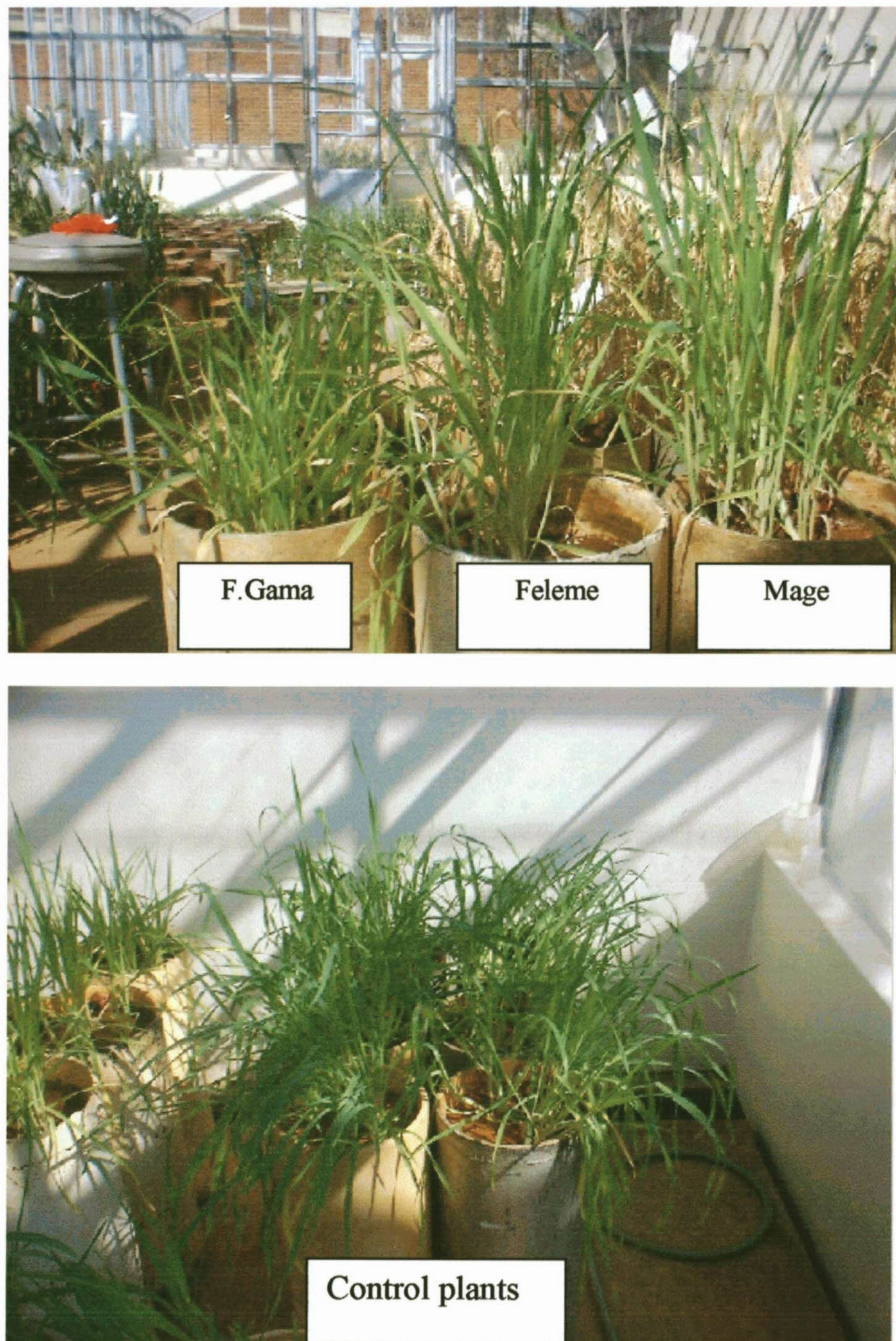

Figure 7.1. Effect of three weeks of waterlogging on growth of tolerant and susceptible barley landraces evaluated in a greenhouse, 2002.

Table 7.1. Effect of waterlogging on growth of barley landraces waterlogged at three to four leaf stage for three weeks in a greenhouse, 2002.

Treatments	Eh (mV)	TTN	TPP	LNP	SDM	SDMP	SDMT (mg)	RDM (g)	S : R	GRPP (mg)	GRPT (mg)
					-----------g---------						
Control (C)	387	32	8	15	9.04	2.26	285	3.59	2.7	129	16
Waterlogged (W)	129	20	5	12	4.48	1.12	234	1.88	2.4	56	11
LSD ($P \leq 0.05$)	***	***	***	***	***	***	**	***	NS	***	**
Feres Gama (C)	390a	32a	8a	15ab	8.16a	2.04a	257b	3.65a	2.5	109b	13b
Mage (C)	390a	31a	8a	16a	9.32a	2.33a	308a	3.53a	2.7	134ab	17a
Feleme (C)	382a	33a	8ab	16a	9.65a	2.41a	292ab	3.61a	2.9	144a	17a
Feres Gama (W)	116b	25b	7b	13bc	4.82b	1.21b	195c	1.94b	2.5	62c	9b
Mage (W)	126b	17c	5c	12c	4.46b	1.12b	259ab	1.93b	2.4	55c	12b
Feleme (W)	145b	17c	4c	12c	4.18b	1.05b	249b	1.79b	2.4	51c	12b
LSD (P=0.05)	40.9	4.7	1.4	2.0	1.94	0.48	49	1.07	NS	34	3.6
C.V (%)	12.2	14.4	16.9	11.3	22.2	22.2	14.6	30.2	32.0	28.4	20.8

Eh=redox potential, TTN=total number of tillers, TPP=tillers per plant, LNP=leaf number per plant, SDMP=shoot dry matter per plant, SDMT=shoot dry matter per tiller, RDM=root dry matter, S:R=shoot to root ratio, GRPP=growth rate per plant and GRPT=growth rate per tiller. *** & ** = significant at 0.001& 0.01 probability level. NS = non significant at P = 0.05.

7.4.3. Effect on nutrients status in roots and shoots

7.4.3.1. Nitrogen, phosphorous and potassium

Waterlogging reduced the concentration of N on average by 32 % compared to its concentration in the free drainage plants and the effect was highly significant (Table 7.2). The effect of waterlogging on N concentration depended on landraces. The decline was very high in the susceptible landrace Feres Gama(37) and Feleme(68). N concentration in the tolerant landrace Mage(07) was on average 13 % higher than in the other two landraces. The proportion of N concentration in waterlogged to free drainage plants was 67.67 %, 64.01 %, and 73.69 % for Feres Gama(37), Feleme(68) and Mage(07), respectively implicating a less severe effect on Mage(07). Root N concentration did not vary between waterlogging treatments and the response of landraces was similar although there was an increasing trend of N concentration in roots of waterlogged plants compared to the free drainage plants. Shoot N concentration accounted for 69 % and 58 % of the total for the free drainage and waterlogged plants, respectively. Increased N concentration in roots of waterlogged plants and a decrease of N concentration in a susceptible variety has been reported for wheat (Huang et al., 1995) although no reports have been found for barley. Reduced N, P and K concentrations were indicated, however, due to short term waterlogging of 13 to 14 days

old barley seedlings (Leyshon & Sheard, 1974; Drew & Sisworo, 1979) the greatest reduction being for P (Leyshon & Sheard, 1974). Waterlogging drastically decreased N uptake on average from 254.07 mg per pot in the free drainage plants to 83.95 mg per pot for the waterlogged plants. There was no apparent difference between landraces for N uptake under waterlogged conditions although the uptake by Feleme(68) was less on average by 10 % than the uptake by the other two landraces (Table 7.3).

The concentration of phosphorous (P) was significantly different (P=0.05) between landraces under waterlogged conditions the effect being higher on the susceptible landrace Feres Gama(37) compared to Mage(07) and Feleme(68) (Table 7.2). The concentration of P for Mage(07) and Feleme(68) under waterlogged conditions were not significantly different from that in the corresponding free drainage plants while there was a 37 % reduction for Feres Gama(37) compared to the control. Although the difference was not significant, P uptake under waterlogged conditions was less in Feres Gama (37) compared to Mage(07) and Feleme(68). The difference between landraces for P concentration in the roots is similar in trend to that of N concentration in roots. There was no difference in P uptake by roots between the free drainage and waterlogged treatments and between landraces (Table 7.3). Although waterlogging had a significant negative effect on K concentration in the shoot, no variation was noticed between landraces. K concentration in the roots did not vary between landraces because of waterlogging. However, K concentration in the roots of waterlogged Feres Gama(37) was much higher than the free drainage. The required concentration of nutrients for barley at the onset of shooting, which corresponds well to this experiment, is N=2.50-5.00 %; P=0.30-0.60 % and K=3.50-5.50 % and at mid shooting, the required concentration for N, P and K is 2.30-3.80 %, 0.25-0.50 % and 3.30-4.50 %, respectively (Bergman, 1992). The concentration of N in Feres Gama(37) and Feleme(68) under waterlogged conditions was below the required range while it was close to the required range for Mage(07). Moreover, P concentration in Mage(07) and Feleme(68) is well within the acceptable range but at the lower margin for the susceptible landrace Feres Gama(37). All landraces had K concentration below the acceptable range under waterlogged conditions.

It was noted that soil waterlogging virtually arrests ion uptake by unadapted roots with pronounced lowering of the average concentration of ions in the leaves, due to

remobilisation from older to younger leaves (Drew & Sisworo, 1979; Trought & Drew, 1980b,c). For example, in barley it was reported that the concentrations (μmol g^{-1}dw) of total N, P and K in leaves had declined, respectively to 75 %, 69 % and 77 % of aerated controls in just 48 h and after 6 days with the corresponding ratios of 37 %, 34 % and 43 % (Drew & Sisworo, 1979).

Table 7.2. Nutrient concentration in the shoots and roots of barley landrace lines waterlogged at three to four leaf stages for three weeks in a greenhouse experiment, 2002.

Treatments	N	P	K	Ca	Mg	Cu	Fe	Zn	Mn
	------	------	%	------	------	------	ppm	------	------
Shoots									
Control (C)	2.768	0.550	4.240	0.441	0.222	6.53	69.60	33.00	59.47
Waterlogged (W)	1.894	0.453	2.863	0.639	0.288	3.63	138.63	13.58	131.3
LSD (P≤0.05)	**	*	*	***	*	**	***	***	***
F.Gama (C)	2.555ab	0.520a	4.450a	0.461bc	0.235ab	5.5ab	85.3b	35.13a	68.1c
Mage (C)	2.809a	0.560a	3.860ab	0.396c	0.192b	6.6a	54.6b	30.88a	55.3c
Feleme (C)	2.940a	0.570a	4.410a	0.466bc	0.238ab	7.5a	68.9b	33.00a	55.0c
F.Gama (W)	1.729c	0.330b	2.620c	0.585ab	0.281a	3.1b	139.8a	11.25b	102.0b
Mage (W)	2.070bc	0.530a	2.980bc	0.686a	0.307a	4.0b	138.5a	15.75b	145.4a
Feleme (W)	1.882c	0.500a	2.990bc	0.646a	0.277a	3.8b	137.6a	13.75b	146.6a
LSD (P=0.05)	0.654	0.140	1.092	0.125	0.080	2.48	44.25	8.95	31.74
C.V (%)	21.66	21.59	23.72	17.88	24.35	37.69	32.80	29.67	25.67
Roots									
Control (C)	1.208	0.230	0.583	0.1213	0.142	13.00	6341.6	43.08	110.75
Waterlogged (W)	1.397	0.363	0.670	0.066	0.138	13.56	7503.6	42.18	215.03
LSD (P≤0.05)	NS	***	NS	NS	NS	NS	NS	NS	***
F.Gama (C)	1.104	0.19c	0.54	0.097	0.121	13.75	6115.0	47.75	106.75c
Mage (C)	1.279	0.25c	0.69	0.106	0.137	12.50	5997.5	41.25	109.75c
Feleme (C)	1.240	0.25c	0.52	0.167	0.167	12.75	6912.5	40.25	115.75c
F.Gama (W)	1.433	0.34b	0.78	0.059	0.137	14.25	6755.0	42.00	198.25b
Mage (W)	1.331	0.35b	0.62	0.065	0.134	12.75	7430.0	40.00	192.00b
Feleme (W)	1.428	0.45a	0.61	0.075	0.143	13.70	8326.0	44.55	254.85a
LSD (P=0.05)	NS	0.076	NS	NS	NS	NS	NS	NS	45.09
C.V (%)	21.83	19.40	22.12	72.55	30.78	17.88	21.48	22.65	21.36

Means followed by the same letter in a column are not significantly different.

*= significant at P=0.05

**= significant at P=0.01

***= significant at P=0.001

NS=none significant.

Table 7.3. Nutrient uptake (mg/pot) by the shoots and roots of barley landrace lines waterlogged at three to four leaf stage for three weeks in a greenhouse experiment, 2002.

Treatments	N	P	K	Ca	Mg	Cu	Fe	Zn	Mn
				Shoots					
Control (C)	254.067	92.291	376.78	39.899	19.93	0.0604	0.6230	0.2979	0.537
Waterlogged (W)	83.947	20.196	127.09	28.485	13.05	0.0163	0.6186	0.0611	0.588
LSD (P≤0.05)	***	***	***	*	*	**	NS	**	NS
F.Gama (C)	205.334b	42.665a	355.78a	38.142	19.229	0.0457ab	0.6930	0.2948a	0.583
Mage (C)	260.097ab	51.131a	357.26a	36.317	17.511	0.0599a	0.4981	0.2819a	0.506
Feleme (C)	296.769a	54.078a	417.32a	45.240	23.044	0.755a	0.6780	0.3169a	0.523
F.Gama (W)	83.948c	16.029b	125.81b	28.372	14.199	0.0153b	0.6776	0.0553b	0.493
Mage (W)	89.728c	23.764b	130.78b	30.417	13.617	0.0178b	06052	0.0703b	0.663
Feleme (W)	78.165c	20.794b	124.70b	26.667	11.416	0.0157b	05731	0.0577b	0.609
LSD (P=0.05)	89.055	12.709	78.13	NS	NS	0.031	NS	0.107	NS
C.V (%)	40.65	28.22	23.92	30.74	35.23	63.19	47.20	45.87	30.55
				Roots					
Control (C)	41.429	8.315	21.705	3.857	5.157	0.048	23.291	0.158	0.406
Waterlogged (W)	26.293	7.064	12.701	1.258	2.595	0.026	14.407	0.079	0.403
LSD (P≤0.05)	**	NS	**	***	**	**	*	**	NS
F.Gama (C)	35.939ab	7.371	21.123ab	3.153a	4.535ab	0.053	23.602	0.181a	0.41
Mage (C)	45.186a	8.653	24.537a	3.718a	4.841ab	0.044	21.178	0.146ab	0.39
Feleme (C)	43.163a	8.920	19.456abc	4.701a	6.095a	0.047	25.092	0.146ab	0.42
F.Gama (W)	27.858b	6.611	15.069bcd	1.140b	2.660b	0.028	13.301	0.082bc	0.38
Mage (W)	25.735b	6.594	11.956cd	1.271b	2.560b	0.025	14.091	0.078c	0.38
Feleme (W)	25.294b	7.986	11.078d	1.365b	2.565b	0.024	15.831	0.078c	0.45
LSD (P=0.05)	12.506	NS	8.376	1.72	2.693	NS	NS	0.067	NS
C.V (%)	28.49	32.96	37.56	51.89	53.61	42.52	41.77	43.99	38.07

Means followed by the same letter in a column are not significantly different.
* = significant at P = 0.05
** = significant at P = 0.01
*** = significant at P = 0.001
NS = non significant

7.4.3.2. Calcium and Magnesium

The concentration of calcium (Ca) and magnesium (Mg) in waterlogging conditions increased on average by 45 % and 30 %, respectively, compared to the values of the free drainage plants but genotypic differences were not evident. Unlike in the shoots, the concentration of Ca in roots showed a decrease of 46 %. The concentration of Mg in roots was not affected by waterlogging. Mean uptake of Ca and Mg were also significantly influenced by waterlogging but in the opposite direction to the concentration. Hence, the uptake in the free drainage plants is greater on average by 40 % and 53 %, respectively for Ca and Mg than in the waterlogged plants. No difference was noticed between landraces for uptake in the waterlogging and free drainage conditions. Glinski & Stepniewski (1985) also noticed that Ca and Mg content in plants are less dependent on root anoxia than that of N, K and P and this was confirmed by

Harris & Bavel (1957). Trought & Drew (1980b) also observed that the uptake of these elements under conditions of root waterlogging was less impeded than that of N, K and P. A decrease in Ca and Mg concentration in shoots of free drainage plants can be contrasted to the case where a nutrient element is applied to a crop and the yield or biomass of the crop increases but on chemical analysis the average concentration of the element in the plant tissue is lower than a deficient control plant (Steenbjerg & Jacobsen, 1963). Such a result could be surprising because of the implicit assumption that the adequacy of the plants' supply of a given nutrient is directly related to the tissue concentration of that nutrient (Martin & Matocha, 1973). In this experiment, the physiological explanation for a decrease of Ca and Mg concentration in the free drainage plants is that the relative rate of dry matter accumulation in the control plants increased more rapidly than the rate of nutrient accumulation resulting in lower final concentrations which is known as dilution effects (Jarrell & Beverly, 1981). Therefore, even though the concentrations of Ca and Mg in the shoots of control plants seems decreased, the total accumulation, as calculated by the product of concentration and dry matter yield, were significantly increased.

7.4.3.3. Zinc and Iron

Pronounced effects of waterlogging were observed for zinc (Zn) and iron (Fe) concentration in the shoot. The concentration of Zn in shoots of the control plants was more than two fold than in the waterlogged plants and its uptake was reduced four fold. Contrary to this the concentration of Fe in shoots of free drainage plants was less by almost two fold compared to that in the waterlogged plants. Although differences were not significant, shoot Zn concentration in waterlogged Mage(07) exceeded Feres Gama(37) by 26 % while the concentrations of Fe in shoots of Feres Gama(37) were higher than in Mage(07) and Feleme(68) under free drainage conditions but no difference was detected under waterlogged conditions. Neither Zn nor Fe concentrations in the roots varied due to waterlogging and the responses of landraces was also similar. However the uptake of Zn by roots was significantly ($P = 0.005$) reduced because of the waterlogging effect.

7.4.3.4. Manganese and copper

Manganese (Mn) concentration increased significantly in shoots and roots of waterlogged plants and decreased in free drainage plants. The uptake of Mn was not

influenced by waterlogging treatment, however. Differences among landraces for Mn concentration in shoots of waterlogged plants was highly significant, the values for Mage(07) and Feleme(68) exceeding that of Feres Gama(37). Landraces did not differ in uptake of Mn despite the significant differences in concentration. This result contradicts that of Huang et al. (1995) who reported an increased concentration of Fe and Mn in the shoots of the sensitive wheat cultivar compared to the tolerant cultivar. Increased concentrations of Fe and Mn in shoots due to waterlogging are in agreement with that of Huang et al. (1995), however. These two nutrients are expected to increase in waterlogged soils because the ferric form of Fe and the manganic form of manganese are converted to the more reduced and soluble ferrous and manganous forms (Ponnamperuma, 1972) which are more readily taken up by roots (Jackson & Drew, 1984) and could lead to a toxicity problem (Huang et al., 1995).

Overall, the reduction of N, P, Ca, Mg and Zn concentration was lower in the tolerant landrace Mage(07) than in the sensitive Feres Gama(37). Inhibition of nutrient uptake and transport by waterlogging could be due to the restriction of root growth (Pezeshki, 1994), the inefficiency of anaerobic metabolism in providing adequate energy for active ion transport (Trought & Drew, 1980c) and increased permeability of cell membranes in roots (Jackson & Drew, 1984). Hence, the lower reduction of the concentration of N, P, Ca, Mg and Zn in shoots of the tolerant Mage(07) compared to the sensitive Feres Gama(37) in situations of waterlogging might be due to the latter two factors, since root growth under waterlogged condition, as judged from the dry matter accumulation, did not vary between landraces.

7.5. Conclusions

After three weeks of waterlogging, soil redox potential decreased from 387mV in the free drainage pots to 129mV in the waterlogged pots. Total number of tillers and tillers per plant did not differ between landraces for free drainage treatment but significantly reduced that of Mage(07) and Feleme(68) under waterlogged conditions. No variation was detected between landraces for number of leaves, however. The reduction in total number of tillers per pot of Mage(07) and Feleme(68) compared to the respective free drainage plants was 43 % and 48 %, respectively while it was only 23 % for Feres

Gama(37). Total shoot dry matter accumulation under waterlogged condition was comparable for all three landraces in spite of the largest number of tillers produced by Feres Gama(37) indicating that Feres Gama(37) achieved comparable shoot dry matter accumulation to Mage(07) and Feleme(68) at the expense of increased number of tillers. Tillers produced by Feres Gama(37) had significantly (P=0.05) less dry matter than Mage(07) and Feleme(68). Although shoot and root dry matter accumulation were significantly reduced due to waterlogging, the shoot : root ratio remained unaffected.

Concentrations of N, P, K, and Cu in shoots of waterlogged plants decreased significantly the effect being less for the tolerant landrace Mage(07) than for Feleme(68) and Feres Gama(37). Moreover, the uptake of N, P, K, Ca, Mg, Cu and Zn by shoots was significantly reduced because of the waterlogging effect. N and P concentrations in the tolerant landrace Mage(07) were higher by 20 % and 61 %, respectively over that of the susceptible landrace Feres Gama(37) although the difference was significant (P=0.05) only for P. Moreover, P uptake by Feres Gama(37) was lower than for Mage(07) and Feleme(68). Nutrient concentrations in roots did not show apparent differences due to waterlogging except for P and Mn. Significant differences were observed for nutrient uptake except that of P and Mn, however. To summarize, differences between the susceptible and tolerant landraces in response to waterlogging were largely due to less dry matter accumulation of the tillers and reduction of growth in the susceptible landrace compared to the tolerant landrace. Moreover, apparent differences were noticed in P concentration and uptake between the tolerant and susceptible landraces, the effect being less for the tolerant landrace than for the susceptible landrace Feres Gama(37). Difference in N concentration of shoots between Feres Gama(37) and Mage(07) was also observed although the magnitude was not comparable to that of P.

CHAPTER VIII

ESTIMATES OF THE BREEDING POTENTIAL OF BARLEY LANDRACES FROM NORTH SHEWA IN ETHIOPIA UNDER WATERLOGGED AND FREE DRAINAGE CONDITIONS

8.1. Abstract

Estimates of genetic parameters are useful since they provide information on the inheritance of characters and help to predict the value of crosses. If the value of crosses cannot be predicted, many crosses need to be made which results in each cross having a small population size, fewer progenies in later generations and a lower probability of recovering good genotypes from each cross. The objectives of this study were therefore i) to estimate genetic parameters from diallel crosses involving five inbred lines: Feres Gama(37), Feleme(68), Mage(07), 1153(28) and 1182(44) that vary for different agronomic characters and ii) to determine the breeding value of the parents so that the progeny performance from crosses involving the best parents could be predicted. Data for agronomic characters were obtained from parents and F1 progenies evaluated in a greenhouse under waterlogged and free drainage conditions. The results highlighted the importance of additive gene action for spike length, number of seeds spike^{-1} and grain yield spike^{-1} under free drainage conditions and for days to heading and days to maturity at both treatment levels. Both additive and non-additive gene action were important in the control of grain yield under free drainage conditions. By contrast, estimates of genetic parameters for yield and yield components (except spike length) were very low or negative under waterlogged conditions. Among the parents, Feres Gama(37) and 1153(28) contributed the highest positive GCA effects and comparable SCA variances for yield and yield components under free drainage conditions. Hence, these parents shall be tested thoroughly in order that maximum use of their superior combining ability can be made in future crossing programs for environments free of waterlogging stress. A separate crossing and selection program is suggested for the respective environmental conditions if resources permit.

8.2. Introduction

Pure line selection within locally adapted germplasm is a promising avenue and one of the easiest, ancient and cheapest methods of improvement (Ceccarelli & Grando, 1996; Lakew et al., 1997). However, pure line selection within landraces is only a short-term strategy and, in the long-term, the best pure lines should be used in the crossing program either with other pure lines from landraces or with non-landrace material so as to cope with the unpredictable variability of abiotic stresses (Ceccarelli & Grando, 1996). To this effect, estimates of genetic parameters for quantitative traits are very useful since they provide information on the inheritance of such traits and help to identify appropriate breeding methods (Dudley & Moll, 1969; Muehlbauer et al., 1995). Such parameters include the subdivision of genetic variance into parameters resulting from additive, dominance and epistatic effects of genes (Muehlbauer et al., 1995; Falconer & Mackay, 1996). The additive component of genotypic variance is very important because it is the chief source of resemblance between relatives, and also the chief determinant of observable genetic properties of the population and of the response of the population to selection (Dabholkar, 1992; Falconer & Mackay, 1996). Therefore, the effectiveness of selection is based on the utilization of additive gene effects (Sprague, 1966) and should be utilized as fully as feasible before undertaking an expensive and time consuming crossing program.

Methods are available for the partitioning of either means or variances that provide information as to the presence or absence of genetic variability and, in addition, provide information on the type of gene action involved (Sprague, 1966; Fehr, 1987; Dabholkar, 1992). Estimation of additive and dominance variances can be obtained through use of a nested design or from diallel crosses (Sprague, 1966). Information from diallel crosses can be used to characterize crossing relationships among a group of varieties or lines with the goal of identifying crosses which would be expected to be good source material for selections (Matzinger, 1963). If the major portion of the variance is additive or additive x additive type of epistatic variance, the breeder can identify superior inbred families and continue inbreeding to homozygosity. Besides, if the variance is primarily of the additive type, that is, if the

parents have a high degree of general combining ability, superior selfed families can be identified on the basis of their crossbred performance and be incorporated into a varietal development program (Baker, 1978)

An important point in estimating combining ability and genetic parameters is the environment in which the test of the progenies was carried out (i.e. stress vs optimum). Gouis et al. (2002) reported differences in general combining ability effects of parents when evaluated under low and high levels of nitrogen and concluded that results obtained at a high N level would not allow identification of parents and that specific experiments at low N level will be necessary. The assumption that in high yielding conditions there is more efficient control of environmental variation, better expression of genetic differences, and hence higher heritabilities than in stress environments (Roy & Murty, 1970) was also argued and it has been shown that it is not always true that heritability is higher in high-yielding than in low-yielding environments (Singh et al., 1993; Ceccarelli, 1994, 1996). Shibin et al. (1996) for instance, observed high heritability (71.5 %) of waterlogging tolerance from analysis of F1 diallel crosses of common wheat which negates the view that heritability is low in stress environments. Moreover, it was noted that measurements made on the same genotypes in two different environments should be regarded as separate and the relative effectiveness of selection strategies depends on the genetic correlations between performances in the two contrasting environments (Falconer, 1981). Hence, the genetic correlation coefficient has to be considered before deciding which the optimum environment for selection is (Ceccarelli, 1994) because partial differences in alleles that control high grain yield in high-yielding and low-yielding environments were also indicated (Ceccarelli et al., 1992).

In Ethiopia, a modest hybridisation program on barley was started in 1968 and up till 1996, 378 crosses were evaluated with the aim to develop high yielding varieties with resistance to scald, net/spot blotches and lodging (Gebre et al., 1996). Although not aggressive, crossing and evaluation of progenies targeted for specific environments was also carried out at Bedi plateau in west Shewa for low soil fertility and low pH and for frost and waterlogging at Sheno in north Shewa. Unfortunately, all crossing experiments were not designed in ways to allow estimates of combining abilities and genetic variances from a landrace based

crossing programs so that information in this regard is not available. The lack of such information will not permit the identification of superior varieties to be used as parents for hybridisation and also pinpoint cross-combinations likely to yield desirable segregates (Dabholkar 1992; Witcombe & Virk, 2001). The objectives of this study were therefore i) to estimate genetic variances, heritability and genetic correlation of characters from crosses between adapted inbred barley landrace lines selected based on their merits and ii) to determine the breeding value of the parents under contrasting environments (free drainage vs waterlogged) so that the progeny performance from crosses involving the best parents could be predicted.

8.3. Materials and methods

8.3.1. Plant materials

Five parents (Feres Gama(37), Feleme(68), 1153(28), 1182(44) and Mage(07) were selected based on their agronomic attributes and differences in response under waterlogging stress. Feres Gama(37) has long spikes (7.8 cm to 8.4 cm), white seeds, gives very good yield but it is late maturing and takes about 88 to 91 days to heading and 142-145 days to mature. Feleme(68) has relatively short spikes (6.5 cm), white seeds, reaches heading in about 80 to 83 days and matures in about 124 days. Line 1153(28) is an early maturing landrace comparable to Feleme(68) and has comparably long spikes to Feres Gama(37) with black seed colour. Mage(07) is a random selection from a local cultivar grown predominantly on low-lying "guie" fields where waterlogging due to excessive rainfall in the main rain season is a problem in north Shewa. It is early in heading and maturity, has irregular spikes with dull white seeds and has good early vegetative growth. Line 1182(44) is a pure line landrace characterized by very short and dense spike, stiff straw and is early as well compared to Feres Gama(37).

8.3.2. Crossing

The five landrace lines were crossed in all possible combinations (excluding reciprocals) to generate 10 F_1 progenies. Crossing was done in an open field at Holetta Research Centre in 2001 (Table 6.1) by hand emasculation with pollination by the approach-cross method.

Table 8.1. Diallel crosses (without reciprocals) among barley landrace lines from north Shewa, Ethiopia.

Parents	Feleme(68)	Mage(07)	1182(44)	1153(28)
Feres Gama(37)	X	X	X	X
Feleme(68)		X	X	X
Mage(07)			X	X
1182(44)				X
1153(28)				_

8.3.3. Experimental design

Two sets of experiments each consisting the five parents and the 10 F_{1s} were set up in a greenhouse. In set I the parents and the crosses were planted in 3 liter size pots perforated at the bottom. Six seeds were planted per pot and thinned later to four uniformly germinated seedlings per pot. The experimental layout was a randomised complete block in four replications. Fertilizer (2: 3: 2 (22) of N: P: K) was applied at the rate of 378.4mg/pot N, 567.6mg P and 378mg K. Pots were watered to field capacity every day for normal growth and development of plants. Insecticide was sprayed, whenever necessary, to control aphids. Set II experiment was conducted with the same parents and crosses to evaluate their response to waterlogging stress. Seedlings were germinated in a similar manner to set I experiment. When seedlings reached three-leaf stage, putting the pots with seedlings in other larger sized pots imposed waterlogging. The larger pots were filled with water until the water level in the pots containing the seedlings reached nearly 10mm above the soil surface. This level of water was maintained for three weeks and thereafter the excess water was drained and plants were allowed to grow until maturity without the waterlogging stress.

8.3.4. Measurements

Days to heading, days to maturity, plant height, spike length, number of seeds spike^{-1}, total productive heads per pot, grain yield per main spike, average kernel mass of main spikes (grain yield per main spike divided by the total number of seeds per main spikes) and grain yield per pot were recorded from the parents and F1.

8.3.5. Statistical methods

8.3.5.1. Analysis of combining ability

Analysis of combining ability was carried out according to Griffing's (1956b) method II (parents and F_1 progenies without reciprocals) and Model I (where genotypes are considered as fixed effects). It may be assumed that the landrace lines used as parents are random selections from populations and thus Model II has to be used. This view, however, is not universally shared (Mayo, 1987) and it is only from the statistical geneticists point of view that variance of combining ability can be considered as population parameters (Sprague, 1966). The breeder is more interested in gene action within a given set of selected inbred lines for which inference is going to be made and hence Model I is preferred to Model II for this experiment. Therefore, the statistical analysis method of Griffing (1956b) as detailed for method II Model I (Table 6.2) was applied. Model I for combining ability analysis is given as:

$$Xij = \mu + gca_i + gca_j + sca_{ij} + 1/bckl\sum\sum ijk\ell$$

Where $ij=1,...p$, $k=1,...b$; $\ell=1,...c$; μ = population mean; gca_i (gca_j) is the general combining ability effect of the $i^{th}(j^{th})$ parents; sca_{ij} is the specific combining ability effect for the cross between the ith and j^{th} parents such that $sca_{ij}=sca_{ji}$ and $eijk\ell$ is the environmental effect associated with the $ijk\ell$th individual observation or simply an error term. The analyses of combining abilities were performed using the Agrobase 2000 computer program.

Table 8.2. The partitioning of the genotype sum of squares in a diallel cross analysis (Method II, Model I).

Source	df	Sum of squares	Mean squares	Expectation of mean squares
General combining ability (GCA)	P -1	S_g	M_g	δ^2+(P+2) (1/P-1)$\sum g_i^2$
Specific combining ability (SCA)	P (p-1)/2	S_s	M_s	δ^2 +[2/P(P-1)]$\sum_i \sum_{ij}^2$
Error	m	S_e	M_e'	δ^2

The ratio of mean square components associated with GCA and SCA effects were calculated according to Baker (1978) to estimate the relative importance of GCA in explaining progeny performance. Statistical testing for GCA effects of parents was done as S.E (Gi) x 1.96 and differences between parents for GCA effects was done as S.E (G_i-G_j) x 1.96. Testing the significance of differences for SCA effects of corsses with one common parent was done as S.E (S_{ij}-S_{jk}) x 1.96 and S.E (Sij-Skl) x 1.96 for crosses with no parent in common (Dabholkar, 1992).

8.3.5.2. Estimation of variance components

Variance of GCA (δ^2gca) was calculated as *(MSgca - MSsca)/n+2* while variance of SCA (δ^2sca) as MSsca-MSe where MSgca, MSsca and MSe stand for mean square of the GCA, SCA, and error, respectively and n is number of parents (Griffing, 1956b). Then, the additive genetic variance (δ^2_A) is twice the GCA variance (2δ^2gca) while the dominance variance (δ^2_D) is the δ^2sca. The total genetic variance (δ^2_g) was calculated as $\delta^2_g = \delta^2_A + \delta^2_D$ and the phenotypic variance (δ^2_p) = $\delta^2_g + \delta^2_e$. The GCA and SCA effects were also used to calculate the estimates of GCA and SCA variances associated with each parent, $\delta_{\hat{g}i}^2$ and $\delta_{\hat{s}i}^2$, respectively according to the method suggested by Griffing (1956b).

8.3.5.3. Estimation of heritability (h^2)

Determination of heritability is one of the first objectives in the genetic study of a metric character. The extent to which individuals' phenotypes are determined by the genotypes is called broad sense heritability *(h^2_b)* and is expressed as the ratio the genotypic variance (δ^2_g) to phenotypic variance (δ^2_p). Hence $h^2_b=\delta^2_g/\delta^2_p$. The

extent to which phenotypes are determined by the genes transmitted from the parents is called narrow sense heritability (h^2_n) and is obtained as the ratio of additive genetic variance (δ^2_A) to phenotypic variance (δ^2_p) expressed as $h^2_n = \delta^2_A / \delta^2_p$ (Falconer and Mackay, 1996).

8.3.5.4. Correlation analysis

A correlation, be it phenotypic, genetic or environmental, is the ratio of the appropriate covariance to the product of the two standard deviations (Falconer &Mackay, 1996) and were computed as follow:

Phenotypic correlation $(r_p) = \sqrt{Covxy/(\delta^2 p_x \delta^2 p_y)}$

where the phenotypic covariance *(CovP)* can be written as $CovP = rp\delta P_x \delta P_y$

Genetic correlation $(r_A) = \sqrt{Covxy/(\delta^2 g_x \delta^2 g_y)}$

$\delta^2 p_x$ and $\delta^2 p_y$ imply the phenotypic variances of characters x and y, respectively and $\delta^2 g_x$ and $\delta^2 g_y$ are the respective genotypic variances. The fact that genetic correlation coefficients are derived from variance and covariance analyses and are not directly estimated makes the test of significance more complex than that of phenotypic or environmental correlation coefficients (Hebert et al., 1994). However, test of significance was performed following Steel and Torrie (1980) as $t = r/\sqrt{(1-r^2)/n-2)}$ where t = Student's t value at n-2 degrees of freedom and n is the number of observations.

8.3.5.5. Estimation of heterosis

Heterosis or hybrid vigour is defined as the difference between the hybrid and the mean of the two parents (Falconer & Mackay, 1996). This type of heterosis is usually called mid-parent heterosis (MPH) which is a deviation of the offspring from mid parent value often expressed as percentage of mid-parent value. Hence, *MPH* is expressed as $[(MF_1-MP)/MP]\ x100$ where MF_1 is the mean value of F_1 cross and MP is the mean value of the two parents. High-parent heterosis (useful heterosis) was calculated from the mean value of the F_1 cross and high-parent value for each character as $HPH = [(MF_1-HP)/HP]\ x\ 100$. A t-test was used to determine

if F_1 cross means were statistically different from mid-parent values as $t = (F_{1ij} - MP_{ij}) / \sqrt{3/8\delta^2 e}$ where F_{1ij} is the mean of the F_1 cross, MP_{ij} is the mid-parent value and $\delta^2 e$ is estimate of error variance (Wyne et al., 1970).

8.4. Results and discussion

8.4.1. Agronomic performance of F1 and parents

Waterlogging remarkably delayed days to heading by 11 to 26 days and on average by 18 days (Table 8.3). The effect was very pronounced on all progenies involving the susceptible parent Feres Gama(37). Accordingly, the difference in days to heading under free drainage and waterlogged conditions of crosses involving this parent was very high. The mean days to heading pooled over parents and progenies was 82 days under free drainage conditions while it was 99 days under waterlogged conditions. The effect was comparatively less on days to maturity. The mean difference in maturity of progenies and parents between control and waterlogged treatments was almost a week.

Although plants under waterlogged conditions had delayed heading and maturity days, they achieved almost equivalent plant height to those of the plants in the free drainage experiment. This was probably because under waterlogged conditions productive tillers were reduced significantly and the surviving tillers might have taken advantage of reduced competition effects for available nutrients that allowed recovery and growth maintenance. Hence, at the end, spike length, number of seeds spike^{-1} and grain yield spike^{-1} of the waterlogged plants was comparable and even in some cases greater than plants in the free drainage experiment. There was a marked difference for grain yield, however, and this was expected because waterlogged and free drainage plants had apparent differences in total productive tillers. The difference in grain yield spike^{-1} between the free drainage and waterlogged plants of Feres Gama(37) is wider (1.24g spike^{-1}) than for Mage(07) that showed a mean difference of only 0.21 g spike^{-1}. Similarly, number of seeds spike^{-1}and grain yield pot^{-1} of the susceptible Feres Gama(37) decreased by 22 and 3.84 g, respectively while the corresponding values for the tolerant Mage(07) was only 5 and 2.0 which indicates differences in the relative sensitivity of the landraces to waterlogging

stress. Moreover, all crosses involving the tolerant parent, Mage(07) had higher grain yield $spike^{-1}$ and grain yield pot^{-1} under waterlogged conditions than all crosses involving Feres Gama(37) as their parent (Table 8.3). The reverse is true under free drainage conditions. This indicates differences between landraces and their progenies in the expression of their genetic potential under drained and waterlogged situations.

Table 8.3. Performance under waterlogged and free drainage growth conditions of parents and F1 progenies from a pot experiment in a greenhouse, 2002.

Parents	DHE			DMA			PLH			SPL			NS/SP			GY/SP			GY/Plot		
	WL	FD	D	WL	FD	D	WL	FD	D	WL	FD	D	WL	FD	D	WL	FD	D	WL	FD	D
F.Gama(37)	116a	102a	14	165	152a	13	88	89	-1	6.4b	7.1a	-0.7	22	44a	-22	1.04	2.28a	-1.24	4.03	7.87	-3.84
Feleme(68)	95bc	81b	15	144	138b	6	79	82	-3	6.2b	5.5c	0.7	38	28c	10	1.80	1.39b	0.40	7.05	8.76	-1.72
Mage(07)	91c	77b	14	144	132c	12	79	83	-4	6.1b	5.7c	0.4	32	27c	5	1.69	1.48b	0.21	5.32	7.32	-2.00
1182(44)	97b	75b	22	142	137bc	5	81	82	-1	3.9c	4.2d	-0.3	26	28c	-2	1.13	1.45b	-0.31	4.64	6.29	-1.65
1153(28)	96bc	81b	16	142	132c	10	82	82	0	6.9a	6.3b	0.6	30	37b	-7	1.73	2.00a	-0.27	5.56	9.23	-3.66
$LSD_{0.05}$	5.8	5.6		4.3	5.2		NS	NS		0.5	0.47		NS	6.25		NS	0.29		NS	NS	
C.V (%)	6.2	7.1		3.0	3.9		7.7	6.1		8.6	8.6		35.0	19.9		33.5	17.8		44.1	18.9	
	DHE			DMA			PLH			SPL			NS/SP			GY/SP			GY/Plot		
F1progenies	WL	FD	D	WL	FD	D	WL	FD	D	WL	FD	D	WL	FD	D	WL	FD	D	WL	FD	D
P1 X P2	110	84	26	144	141	3	79	80	-1	7.4	6.6	0.8	34	42	-8	1.58	2.21	-0.63	5.29	9.16	-3.87
P1 X P3	104	87	17	152	141	11	87	84	3	7.0	6.6	0.4	41	44	-3	1.87	2.01	-0.14	7.49	9.29	-1.80
P1 X P4	106	81	25	151	136	15	78	91	-13	7.1	7.1	0.0	27	47	-20	1.54	2.48	-0.94	6.15	10.25	-4.10
P1 X P5	107	88	19	153	142	11	81	89	-8	6.9	7.6	-0.7	29	41	-12	1.65	2.34	-0.69	7.61	10.82	-3.21
P2 X P3	92	81	11	142	137	5	86	81	5	6.6	5.6	1.0	38	33	5	2.02	1.55	0.47	7.87	8.65	-0.78
P2 X P4	94	78	16	142	133	9	78	79	1	6.4	6.2	0.2	37	31	6	1.63	1.64	-0.01	6.31	7.61	-1.30
P2 X P5	99	81	18	139	143	-4	78	83	-5	6.6	6.6	0.0	29	33	-4	1.63	1.65	-0.02	6.49	9.91	-3.42
P3 X P4	101	79	22	143	132	11	92	-	-	7.1	6.5	0.6	40	33	7	2.22	1.64	0.58	7.90	8.55	-0.65
P3 X P5	92	75	17	138	135	3	84	80	4	6.8	6.5	0.3	41	31	10	2.10	1.73	0.37	8.26	8.14	0.12
P4 X P5	93	79	15	139	131	8	81	85	-4	6.4	6.2	0.2	37	43	-6	2.09	1.97	0.12	8.07	10.57	-2.50
$LSD_{0.05}$	6.9	3.8		5.3	5.2		NS	NS		NS	0.46		NS	7.2		NS	0.37		NS	1.52	

DHE=days to heading; DMA = days to maturity; PLH = plant height (cm); NS/SP = number of seeds $spike^{-1}$; GY/SP = grain yield $spike^{-1}$; GY/plot = grain yield $plot^{-1}$; D = difference; P1 to P3 are symbols representing parents listed in order in the table.

8.4.2. Combining ability effects

Under waterlogging conditions, the analysis of variance showed significant mean square values of general combining ability (GCA) for days to heading, days to maturity, number of seeds spike^{-1} and grain yield spike^{-1}, but not the specific combining ability (Table 8.4) implying additive genetic mechanisms might be important in determining these characters. Consistent with the free drainage treatment, GCA for days to heading, days to maturity, and grain yield spike^{-1} were significant under conditions of waterlogging. Both GCA and SCA mean square values were highly significant for spike length under both treatment levels, however, suggesting the importance of both additive and dominant gene action for this character. However, a GCA/SCA ratio higher than unity demonstrates that this character is predominantly under the control of additive gene action. In the free drainage treatment, grain yield appeared to be determined both by additive and dominant gene action as observed from the low GCA/SCA ratio.

Several combining ability studies in barley (Hockett et al., 1993; Bhatnagar & Sharma, 1995; Schittenhelm et al., 1996; Bhatnagar & Sharma, 1997; Hanifi & Gallais, 1999) indicated that GCA effects are more important in determining grain yield and yield components in environments free of stress. Phogat et al. (1995b), however, reported that both GCA and SCA are important for yield and yield components. A genetic study for tolerance to waterlogging in barley is lacking and a comparison with other studies is not possible. Based on the result from the free drainage experiment, it can be deduced that in absence of significant SCA effects the performance of the crossed progenies could be predicted based on GCA estimates of the parents because the parents with higher GCA estimates would be expected to produce superior cross bred progenies. In this regard Feres Gama(37) and 1153(28) were found to be good combiners. It was reported, however, that crosses between good general combiners would not always result in good F_1 combinations (Wells & Lay, 1970; Shrivastava & Seshu, 1983).

Table 8.4. Combining ability analysis of F_1s and parents in a 5 x 5 diallel crosses of barley landrace lines evaluated under freely drained (FD) and waterlogged (WL) conditions in a greenhouse, 2002.

Source of variation	Expt.	DF	Agronomic characters						
			DHE	DMA	PLH	SPL	NS/SP	GY/SP	GY
GCA	FD	4	134.511***	92.253***	26.051	1.314***	107.730***	0.361***	1.852*
	WL	4	153.167***	148.853***	16.854	0.877***	39.421	0.173	1.467
SCA	FD	10	10.219	12.680	7.628	0.381***	20.401	0.037	1.497*
	WL	10	10.294	11.155	19.786	0.582***	30.042	0.083	2.040
Residual	FD	42	5.684	6.656	8.203	0.061	12.758	0.034	0.654
	WL	42	12.397	6.961	13.864	0.086	19.252	0.066	1.528
GCA/SCA	FD		13.16	7.270	3.415	3.450	5.280	9.750	1.240
	WL		14.88	13.940	-	1.507	-	2.080	-

Expt.=experiment; FD=free drainage; WL=waterlogged; DHE=days to heading & DMA= days to maturity; PLH=plant height; SPL=spike length; NS/SP=number of seeds Spike^{-1}; GY/SP=grain yield spike^{-1} and GY=grain yield. * and ****= significantly different at P≤0.05 and P≤0.001, respectively.

Table 8..5. General combining ability (GCA) effect of parents and mean performance for agronomic traits of barley landrace lines from evaluation of a diallel cross under free drainage conditions.

Parents	DHE		DMA		PLH		SPL		NS/SP		GY/SP		GY(g/pot)	
	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
F.Gama(37)	102a	7.636**	152a	5.943**	89	2.957	7.1a	0.619**	44a	6.378**	2.27a	0.352**	7.88	0.305
Feleme(68)	81b	-0.900	138b	1.014	82	-2.293	5.5c	-0.252**	28c	-3.121**	1.38b	-0.187**	8.76	-0.016
Mage(07)	77b	-2.150**	132c	-2.628**	83	-0.829	5.7c	-0.152	27c	-3.086*	1.47b	-0.180**	7.32	-0.492
1182(44)	75b	-3.436**	137bc	-2.378**	82	0.386	4.2d	-0.464**	28c	-0.978	1.44b	-0.079	6.29	-0.509
1153(28)	81b	-1.150	132c	-1.950*	82	-0.221	6.3b	0.251**	37b	0.807	2.00a	0.095	9.22	0.680*
$LSD_{0.05}$	5.7		5.2		NS		0.47		6.2		0.29		NS	
C.V(%)	7.1		3.9		6.0		8.6		19.9		17.8		18.9	
Gi		1.579		1.709		NS		0.163		2.366		0.122		0.536
Gi-Gj		2.498		2.703		NS		0.258		3.742		0.192		0.847

Table 8.6. General combining ability (GCA) effect of parents and mean performance for agronomic traits of barley landrace lines from evaluation of a diallel cross under waterlogged conditions.

Parents	DHE		DMA		PLH		SPL		NS/SP		GY/SP		GY(g/pot)	
	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
F.Gama(37)	116a	8.207**	165a	8.214**	88	1.064	6.3b	0.296**	22	-3.028	1.04	-0.222	4.03	-0.674
Feleme(68)	95bc	-1.686	144b	-2.571**	79	-2.007	6.2b	0.039	38	1.614	1.80	0.023	7.05	0.106
Mage(07)	91c	-3.543**	144b	-1.393	79	1.957	6.1b	0.078	32	3.043	1.69	0.185	5.32	0.442
1182(44)	97b	-1.078	142b	-2.107*	81	-0.257	3.9c	-0.607**	26	-0.528	1.13	-0.077	4.64	-0.228
1153(28)	96bc	-1.900	145b	-2.143*	82	-0.757	6.9a	0.193	30	-1.100	1.73	0.091	5.56	0.354
$LSD_{0.05}$	5.8		4.3		NS		0.48		NS		NS		NS	
C.V(%)	6.2		3.0		7.7		8.6		35.0		33.5		44.0	
Gi		2.333		1.748		NS		0.194		NS		NS		NS
Gi-Gj		3.688		2.764		NS		0.307		NS		NS		NS

DHE=days to heading & DMA= days to maturity; PLH=plant height; SPL=spike length; NS/SP=number of seeds $spike^{-1}$; GY/SP=grain yield $spike^{-1}$ and GY=grain yield.

* and **= significantly different at P=0.05 and P=0.01, respectively; NS = none significant.

The patterns of GCA effects of parents for days to heading and days to maturity are similar for the free drainage and waterlogging treatments in that the three early lines, Mage(07), 1182(44), and 1153(28), all had negative GCA effects for days to heading and maturity and Feres Gama(37) had positive GCA effects at both treatments (Tables 8.5 & 8.6). Earliness is a desirable feature and crosses involving these lines are expected to provide on average early heading and maturing progenies regardless of the waterlogging or free drainage treatments. Mage(07) and 1182(44) had negative GCA effects on yield and yield components, however, and are not the desired parents if the aim is to improve grain yield for environments where waterlogging is not a problem. However, yield stability is more important than high grain yield under stress environments and Mage(07) may be the preferred parent because it has consistently higher positive GCA effects for yield and yield components than all other parents under waterlogged conditions. The non-significant GCA mean square values for yield and yield components except spike length put the importance of this line in question, however. Under the free drainage environment, among the early lines, 1153(28) had positive GCA effects for all yield components and implicated the possibility of combining earliness and high grain yield. Feres Gama(37), on the other hand, is very late compared to the other three lines and accordingly demonstrated positive GCA effects for days to heading and maturity. Moreover, the GCA effects of Feres Gama(37) is higher than GCA effects of the other parent lines in all cases and the effects were significant for all characters observed under free drainage condition (Table 8.5).

Generally, under free drainage conditions, Feres Gama(37) and 1153(28) contributed the highest positive GCA effects for yield and yield components (spike length, number of seeds spike^{-1} and grain yield spike^{-1}). They were found to be good combiners for yield and yield components and accordingly the cross between these two parents gave the highest mean spike length, grain yield spike^{-1} and grain yield than all crosses. This cross is also among the top in number of seeds spike^{-1} in the free drainage experiment (Table 8.7). The facts that GCA effects of 1153(28) for number of seeds spike^{-1} and grain yield spike^{-1} were not significant imply, however, that this parent is not as good a combiner as Feres Gama(37) for these characters. The difference between the GCA effects of the two parents for spike length is

significant denoting that both parents are desirable whereas the difference in GCA effects for grain yield is not significant.

Although Feres Gama(37) had significant positive GCA effects for days to heading and maturity, under free drainage experiment, all crosses with this parent showed negative SCA effects for days to heading and maturity except that of Feres Gama(37) x Mage(07) and Feres Gama(37) x 1153(28) which had positive SCA effects for days to maturity (Table 8.7). SCA mean square values were not significant, however, for these characters under both treatment levels. Hence, it is not important to discuss SCA effects. Therefore, restricting the discussion to spike length and grain yield in which both GCA and SCA mean square values were significant in the free drainage experiment (Table 8.4), high and positive SCA effects with improved spike length was observed in crosses of Feres Gama(37) x 1182(44), Feres Gama(37) x 1153(28) and Mage(07) x 1182(44) in which spike length of these crosses is above the high parent of the respective crosses. Higher SCA effects for grain yield were also observed in these three crosses and 1182(44) x 1153(28) in which grain yield was far above the high parent value of the respective parents (Table 8.7). In this of experimental set, some of the crosses which showed significant positive SCA effects for spike length (Feres Gama(37) x 1182(44) and for grain yield (Feres Gama(37) x 1182(44) and 1182(44) x 1153(28) involved one good and one poor general combiner for these characters. According to Singh et al. (1985) such crosses would be expected to produce desirable transgressive segregants if the additive genetic system present in the good combiners (1153(28) and Feres Gama(37) and complementary epistatic effects present in the F_1 act in the same direction to maximize the desirable attributes. Under waterlogging conditions only spike length appeared to have significant SCA mean squares and the highest positive SCA effects were noted for crosses between Feres Gama(37) x Feleme(38), Mage(07) x 1182(44) and Feres Gama(37) x 1182(44). The highest mean spike lengths, among all F1, were also observed from these crosses.

Table 8.7. Mean agronomic performance and specific combining ability effects of F1 progeny from diallel crosses of landrace lines evaluated under free drainage conditions in a greenhouse in 2002.

Crosses	DHE		DMA		SPL(cm)		NS/SP		GY/SP(g)		GY (g/pot)	
	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA
F.Gama(37) x Feleme(68)	84	-4.369	141	-3.190	6.5	-0.107	42	2.976	2.21	0.164	9.15	-0.019
F.gama(37) x Mage(07)	87	-0.369	141	0.952	6.6	-0.107	44	4.190	2.01	0.087	9.29	0.631
F.gama(37) x 1182(44)	81	-5.083	136	-4.298	7.1	0.682*	47	5.583	2.48	0.332	10.25	1.598*
F.Gama(37) x 1153(28)	88	-0.369	142	1.274	7.6	0.439*	41	-2.702	2.34	0.020	10.82	0.983
Feleme(68) x Mage(07)	81	2.167	137	1.131	5.6	-0.285	33	2.690	1.55	0.041	8.65	0.672
Feleme(68) x 1182(44)	78	0.452	133	-3.119	6.2	0.603*	31	-0.667	1.64	0.028	7.61	-0.750
Feleme(38) x 1153(28)	81	0.917	143	6.702	6.6	0.311	33	-0.952	1.65	0.006	7.90	0.355
Mage(07) x 1182(44)	79	2.702	132	-0.274	6.5	0.853*	33	0.798	1.64	0.018	8.55	0.700
Mage(07) x 1153(28)	75	-3.833	130	-2.905	6.5	0.061	31	-2.488	1.73	-0.065	8.14	-0.899
1182(44) x 1153(28)	79	1.452	132	-1.405	6.2	0.125	43	6.905	1.97	0.169	10.56	1.540*
Mean	82		137		6.3		36		1.85		8.83	
$LSD_{0.05}$	6.8		8.6		0.7		10		0.54		2.46	
$LSD_{0.01}$	9.1		11.5		0.9		14		0.72		NS	
C.V (%)	5.82		4.4		7.8		19.8		19.90		19.40	

DHE & DMA= days to heading and maturity, respectively; SPL= spike length; NS/SP=number of seeds per spike; GY/SP=grain yield per spike GY=grain yield; *=significantly different at 0.05 probability.

In the free drainage experiment, GCA and SCA variance estimates associated with each parent (Table 8.8) indicated that Feres Gama(37) and 1153(28) had comparable SCA variances for yield and yield components suggesting that both parents transferred uniformly their potential to improve yield and yield components to their progeny. However, the relatively lower SCA variance for spike length associated with 1153(28) indicated that the potential for improved spike length was transferred better by this parent than Feres Gama(37). Under waterlogged conditions, neither yield nor yield components had significant GCA and SCA mean square values except spike length which was also the case in ordinary analyses of variance. Hence a comparison of GCA and SCA variances associated with each parent would not be fair and results are not presented. A parent with comparatively lower SCA variance for a particular trait is said to transfer its potential uniformly to all the F_1 progeny (Griffing, 1956b; Boghi & Perenzin, 1994). Hence, these parents, Feres Gama(37) and 1153(28), shall be tested thoroughly in order that maximum use of their superior combining ability can be made in future crossing programs for environments free of waterlogging problem.

Table 8.8. Estimates of GCA variance ($\delta_{\hat{g}i}^2$) and SCA variance ($\delta_{\hat{s}i}^2$) of parents for the different agronomic characters from a diallel cross of barley landrace lines evaluated under free drainage conditions in a greenhouse, 2002.

Parents	Variance	DHE	DMA	PLH	SPL	NS/SP	GY/SP	GY
F.Gama(37)	$\delta_{\hat{g}i}^2$	56.788	33.543	6.557	0.367	37.284	0.115	-0.081
	$\delta_{\hat{s}i}^2$	11.276	5.955	7.525	0.186	13.123	0.026	0.871
Feleme(68)	$\delta_{\hat{g}i}^2$	-0.706	-0.746	3.069	0.047	6.341	0.026	-0.174
	$\delta_{\hat{s}i}^2$	4.487	17.596	4.194	0.144	-2.690	-0.013	-0.055
Mage(07)	$\delta_{\hat{g}i}^2$	3.107	5.135	-1.501	0.007	6.119	0.023	0.068
	$\delta_{\hat{s}i}^2$	5.152	-0.871	-1.562	0.234	2.035	-0.018	0.281
1182(44)	$\delta_{\hat{g}i}^2$	10.288	3.883	-2.038	0.201	-2.444	-0.003	0.085
	$\delta_{\hat{s}i}^2$	-3.703	5.646	4.117	0.484	18.139	0.024	1.557
1153(28)	$\delta_{\hat{g}i}^2$	-0.193	2.027	-2.138	0.047	-2.751	0.000	0.288
	$\delta_{\hat{s}i}^2$	2.136	14.547	0.066	0.062	12.187	-0.012	0.989

$\delta_{\hat{g}i}^2$, $\delta_{\hat{s}i}^2$= general combining ability and specific combining ability variance of each parent, respectively.

8.4.3. Estimates of genetic parameters

Genetic parameters of agronomic characters were estimated from F_1 progenies and parents evaluated under free drainage and waterlogging stress. Estimates of the parameters from the respective experiments are presented in Table 8.9 and 8.10. The results from the free drainage experiment elucidated that of the total genotypic variance ($\delta^2g = \delta^2_A + \delta^2_D$), the additive genetic variance portion is very high for all characters except for grain yield in which the δ^2_D is greater than the δ^2_A and spike length that showed comparable values of δ^2_A and δ^2_D. Spike length was the only character that displayed significant SCA mean squares hence relatively higher δ^2_D. However, the additive genetic variance was lower than the dominance variance (δ^2_D) under waterlogged conditions. The fact that the GCA: SCA ratio was relatively higher may lead to the assertion that additive gene action is more important than the non-additive portion in the inheritance of this character. Dabholkar (1992), however, indicated that it is erroneous to conclude that additive or non-additive gene action is predominant on the basis of relative magnitude of significant GCA and SCA mean square values without considering the respective GCA and SCA variances. This is true because the variance of general combining ability is equal to the additive variance and the variance of specific combining ability is equal to the non-additive variance (Falconer & Mackay, 1996). Hence in view of this, it may be assumed that spike length is not under the control of additive gene action under waterlogging stress since the GCA variance (δ^2gca) is lower than SCA variance (δ^2sca) suggesting low genetic advance by selection for this character.

The predominant role of non-additive gene action in the inheritance of grain yield (Kudla & Kudla, 1995; Bouzerzour, & Djakoune, 1997), the importance of additive gene action in determining grain yield spike^{-1} and heading date (Kudla & Kudla, 1995; Esparza-Martinez & Foster, 1998), and number of seeds spike^{-1} (Bouzerzour, & Djakoune, 1997) has been reported in environments free of stress. In this study, although grain yield appeared to be governed both by additive and non-additive gene actions under free drainage conditions, yield components were found to be under the effects of additive gene action that is in harmony with most of the above studies. By contrast, the importance of both additive and non-additive gene actions for yield and yield components (Bhatnagar & Sharma, 1995; Phogat et al., 1995a) and for days to heading and maturity (Singh & Singh, 1990a) have been reported which is in contrast to this experiment under free drainage conditions. Under waterlogging stress, the additive genetic variance (δ^2_A) for days to heading and days to maturity were very high in contrast to their respective dominance variance (Table 8.10) indicating the importance of additive gene actions in the expression of these characters which was consistent with the results from the free drainage experiment.

Table 8.9. Estimates of genetic parameters for seven agronomic characters of F1s from a diallel cross of barley landrace lines evaluated under free drainage conditions in a greenhouse, 2002.

Character	GCA	SCA	δ^2_e	δ^2_{gca}	δ^2_{sca}	δ^2_A	δ^2_D	δ^2_g	δ^2_p	h^2_b	h^2n	PR
DHE	134.511	10.219	5.684	18.404	4.535	36.807	4.535	41.343	47.026	0.88	0.78	0.89
DMA	92.253	12.680	6.656	12.228	6.024	24.456	6.024	30.480	37.136	0.82	0.66	0.80
PLH	26.051	7.628	8.203	2.549	-0.575	5.099	-0.575	4.524	12.727	0.35	0.40	1.13
SPL	1.314	0.381	0.061	0.179	0.320	0.358	0.320	0.678	0.739	0.92	0.48	0.53
NS/SP	107.730	20.401	12.758	13.567	7.643	27.134	7.643	34.777	47.535	0.73	0.57	0.78
GY/SP	0.361	0.037	0.034	0.047	0.003	0.093	0.003	0.096	0.130	0.71	0.72	0.97
GY	1.852	1.497	0.654	0.171	0.843	0.342	0.843	1.185	1.839	0.64	0.19	0.29

Table 8.10. Estimates of genetic parameters for the different agronomic characters of F1s and parents from a diallel cross of barley landrace lines evaluated under situations of waterlogging for three weeks in a greenhouse pot experiment, 2002.

Variable	MSgca	MSsca	δ^2e	δ^2gca	δ^2sca	δ^2_A	δ^2_D	δ^2g	δ^2p	h^2b	h^2n	PR	GCA: SCA
DHE	153.167***	10.294	12.397	20.410	-2.103	40.82	-2.10	38.72	51.11	0.76	0.79	1.05	14.88
DMA	148.853***	11.155	6.961	19.671	4.194	39.34	4.19	43.54	50.49	0.86	0.78	0.90	12.34
PLH	16.854**	19.786	13.864	-0.419	5.922	-0.84	5.92	5.08	18.95	0.27	-0.04	-0.16	-
SPL	0.877***	0.582***	0.086	0.042	0.496	0.08	0.49	0.580	0.67	0.87	0.13	0.15	1.51
NS/SP	39.421	30.042	19.252	1.339	10.790	2.68	3.07	5.75	25.00	0.23	0.11	0.19	-
GY/SP	0.173*	0.083	0.066	0.013	0.017	0.026	-2.10	-2.08	-2.01	1.03	-0.01	0.60	2.08
GY	1.467	2.040	1.528	-0.082	0.512	-0.16	0.02	-0.15	1.38	-0.10	-0.12	-0.47	-

*, *** = significantly different at $P < 0.05$ and $P < 0.001$, respectively. – represents GCA:SSA ratio not calculated because neither GCA nor SCA mean square values were significant.

Estimates of broad sense heritability (h^2b) were in the range of 0.35 for plant height to 0.92 for spike length under free drainage condition and 0.00 for grain yield to 1.03 for grain yield spike^{-1} under waterlogged conditions. Values for narrow sense heritability (h^2n) were in the range of 0.19 for grain yield to 0.78 for days to heading in the free drainage experiment while it was 0.00 for grain yield to 0.79 for days to heading in the case of waterlogging experiment. Heritabilities for the reproductive characters (number of seeds spike^{-1}, grain yield spike^{-1} and grain yield) were very low to moderate in the free drainage experiment whereas both h^2b and h^2n were moderate to very high for the phonological characters for both treatment levels. Higher heritability estimates reported for days to heading (Frey, 1954; Singh & Singh, 1990a; Cai et al., 1993), and number of seeds spike^{-1} (El-Hennawy, 1997) and low heritability for grain yield (Grafius et al., 1952; Cai et al., 1993; Bailey & Wolfe, 1994; Phogat, et al., 1995) in barley are in agreement with results obtained from the free drainage experiment.

It is obvious that heritability in the broad sense may be regarded as an estimate of the upper bound of heritability in the narrow sense since it includes both the additive and non-additive genetic variances. Hence, it should not be preferred if h^2n is available because it is the h^2n which expresses the extent to which the phenotypes are determined by the genes transmitted from the parents (Bos & Caligari, 1995). Moreover, heritability in the narrow sense being the ratio of the additive genetic variance to the phenotypic variance is a scale-independent quantity and plays an important role in predicting the response to selection. In view of this, the very low additive variance compared to the dominance variance and the consequently very low h^2n for grain yield in the free drainage experiment (Table 8.9) imply that genetic advance by selecting for this character is expected to be low. On the other hand, because of the absence of the dominant genetic variance for grain yield per spike, higher h^2n was observed. Hence, it is possible to select barley plants with a desirable grain yield per spike in early generations, and indirect selection for grain yield through selection for grain yield per spike appears to be feasible under free drainage environment. It's predictability (PR=0.97) was also higher compared to all other yield components.

The waterlogged experiment was generally characterized by negative estimates of genetic parameters that were not the case with data from the free drainage experiment. Miller et al. (1958) discussed negative estimates of genetic parameters and attributed it to sampling error and the negative estimates shall be regarded as zero values. Hogarth (1971) indicated that negative estimates of genetic parameters are meaningless, but they should be presented for illustrative purpose, the values being taken as zero, or in order to contribute to the accumulation of knowledge (Dudley & Moll, 1969). Maluf et al. (1983) put his notion, however, that negative value of genotypic variance is most likely the result of low magnitude of genotypic variance in relation to variance of error (δ^2_e) and not because of the non-existence of genetic variation; or because of situations where characters in the parental means are very close so that variance estimates in the hybrid population will be close to zero (Haddad, 1982). Hence, the resulting negative heritability values shall be considered as very low rather than zero.

Lack of precision in an experiment was also considered as a major factor for negative variances. Comstock and Moll (1963) showed that well replicated experiments in time and space would improve precision or repeated experimentation involving the same character in related populations will give estimates, which when averaged, approach a true value (Dudley & Moll, 1969). However, Hogarth (1971) obtained negative estimates of genetic parameters despite the high precision in his experiment as judged by the coefficient of variation and he coined the issue with his view that it is a major problem in quantitative genetic studies. Considering all these views, the negative estimates of genetic parameters observed in this study under waterlogged conditions shall be treated with caution.

8.4.4. Estimates of heterosis

In the free drainage experiment, mid-parent heterosis for days to heading was in the range of -3.1 to -7.8 % in 50 % of the crosses almost all being crosses between Feres Gama(37) and the other early heading and maturing landraces (Table 8.11). It was also negative (-0.9 to -4.9%) in 50 % of the crosses evaluated under waterlogged conditions (Table 8.12) although they are not the same crosses as in the free drainage experiment indicating differences between crosses in expression of mid-parent heterosis at the two treatment levels. Similarly, mid-parent heterosis for days to maturity was negative in 70 % and 90 % of the crosses in the free drainage and waterlogged experiments, respectively manifesting relative earliness of F1 compared to their mid parent values under waterlogged conditions. The differences in days to heading and days to maturity between F_1 and their mid-parent values in the free drainage experiment were negligible, however, except in crosses between Feres Gama(37) x Feleme(68) and Feres Gama(37) x 1182(44) which had headed seven and eight days earlier than their mid-parent values, respectively (Table 8.13). The latter cross was eight days earlier in maturity than the mid-parent value. Similar comparison for the waterlogged experiment revealed a non significant difference for days to heading across all crosses (Table 8.11) while it was significant for days to maturity only for the crosses between Feres Gama(37) x Feleme(68) and Mage(07) x 1153(28) which matured 11 and 7 days earlier than their respective mid parent values. Low-parent heterosis was positive in almost all crosses except Mage(07) x 1153(28) which headed two days earlier than the low parent value and Feleme(68) x 1182(44) which matured four days earlier than the low parent value under free

drainage conditions (Table 8.11). Only Mage(07) x 1153(28) matured 6 days earlier than the low parent value under waterlogged conditions.

Table 8.11. Mid-parent (MP) and high parent (HP) heterosis values of F1s from a diallel cross of barley landrace lines evaluated under free drainage conditions in a greenhouse, 2002.

Crosses	DHE		DMA		SPL		NS/SP		GY/SP		GY	
	MP	HP	MP	HP	MP	HP	MP	HP	MP	HP	MP	HP
F.Gama(37)xFeleme(68)	-7.8	4.6	-2.8	1.8	3.9	-7.4	18.2	-3.4	20.5	-3.2	10.1	4.5
F.Gama(37)xMage(07)	-3.1	12.6	-0.4	7.0	3.9	-6.0	22.5	-0.6	7.0	-11.8	22.4	18.1
F.Gama(37)x1182(44)	-8.7	7.6	-5.4	-0.2	26.1	0.7	31.5	7.4	33.3	8.9	44.7	30.2
F.Gama(37)x1153(28)	-3.7	9.3	0.5	8.2	13.4	7.4	0.0	-7.4	9.5	2.9	26.6	17.3
Feleme(68)xMage(07)	2.7	4.8	1.0	3.4	-0.4	-2.2	18.2	17.1	8.4	5.1	7.6	-1.2
Feleme(68)x1182(44)	0.16	3.6	-3.5	-2.9	26.7	11.7	12.6	12.6	15.9	13.5	1.1	-13.1
Feleme(68)x1153(28)	0.31	0.3	5.8	8.6	11.4	-12.6	0.8	-12.1	-2.4	-17.5	10.1	7.3
Mage(07)x1182(44)	3.6	4.9	-1.5	0.2	31.2	13.9	19.1	18.0	12.0	10.8	25.7	16.9
Mage(07)x1153(28)	-5.2	-3.2	-1.7	-1.9	7.1	1.9	-3.1	-16.1	0.1	-13.7	-1.5	-11.7
1182(44)x1153(28)	1.1	4.6	-1.8	0.0	17.5	-1.9	31.5	14.7	14.4	-1.5	36.2	14.5

Table 8.12. Mid-parent (MP) and high parent (HP) heterosis values of F1s from a diallel cross of barley landrace lines evaluated under waterlogged conditions in a greenhouse, 2002.

Crosses	DHE		DMA		SPL		NS/SP		GY/SP		GY	
	MP	HP	MP	HP	MP	HP	MP	HP	MP	HP	MP	HP
F.Gama(37)xFeleme(68)	3.7	15.7	-7.1	0.0	17.5	17.4	13.3	-12.2	11.3	-10.5	-4.5	24.9
F.Gama(37)xMage(07)	0.0	14.3	-1.9	5.5	12.9	11.1	51.8	10.6	37.5	28.1	60.4	40.7
F.Gama(37)x1182(44)	-0.9	9.3	-1.9	6.3	39.2	12.6	12.5	36.2	42.6	3.8	15.4	32.5
F.Gama(37)x1153(28)	0.9	11.4	-1.3	5.5	4.5	0.0	11.5	-4.6	19.5	-3.3	58.8	36.8
Feleme(68)xMage(07)	-1.1	1.1	-1.4	-1.4	8.2	6.4	8.6	12.2	16.1	0.0	27.3	11.6
Feleme(68)x1182(44)	2.1	-1.1	-0.7	0.0	25.5	3.2	15.6	-9.4	11.6	-2.6	8.0	-10.5
Feleme(68)x1153(28)	3.1	4.2	-3.5	-3.5	1.5	-4.3	-14.7	-9.4	-7.4	-23.6	3.0	-7.9
Mage(07)x1182(44)	7.4	10.9	0.0	0.7	42.0	16.4	37.9	31.3	57.4	25.0	58.6	48.5
Mage(07)x1153(28)	-2.1	1.1	-4.8	-4.2	4.6	-1.4	32.2	21.4	22.8	28.1	51.8	48.5
1182(44)x1153(28)	-4.1	-3.1	-3.5	-2.1	18.5	-7.2	32.1	20.8	46.1	23.3	58.2	45.1

Mid-parent heterosis for yield components (spike length, number of seeds per spike and grain yield per spike) under free drainage was higher than for the phenological characters and was mostly positive. Mid-parent heterosis for spike length of the cross between the two agronomically important lines with good GCA values (Feres Gama-03 x 1153(28) was also fairly high (13.4 %) in free drainage conditions but very low (4.5 %) in waterlogged conditions. The highest value (31 % in free drainage and 42 % in waterlogged condition) for spike length was observed from the

cross Mage(07) x 1182(44), however, followed by Feleme(68) x 1182(44) and Feres Gama(03) x 1182(44) with the respective values of 27 % and 26 % under free drainage and 25.5 % and 39.2 % under waterlogged conditions. The differences in spike length of these crosses from the mid-parent value of the respective crosses were also significant (Table 8.13 and 8.14) at both treatment levels. These three crosses demonstrated high mid-parent heterosis for number of seeds spike^{-1} and grain yield per spike more or less consistently across the two treatments. The highest mid-parent heterosis (31 %) for number of seeds spike^{-1} was revealed by 1182(44) x 1153(28) and Feres Gama(37) x 1182(44) in free drainage treatment while it was 51.8 % in waterlogged treatment by Feres Gama(37) x Mage(07). All crosses between Feres Gama(37) and the other lines and that between 1182(44) x 1153(28) showed the highest values of mid-parent heterosis for grain yield in the free drainage treatment. Yield and yield components generally revealed higher heterosis than days to heading and maturity at both treatment levels.

Self-fertilizing species generally show a small amount of heterosis when crosses are made (Falconer & Mackay, 1996). The purpose of crossing self-pollinated crops is, therefore, not to make use of heterosis for commercial growing but to produce a new set of inbred lines which become differentiated by recombination so that one or more of these recombinant lines can be found which are better than either of the parental lines.

Table 8.13. Comparisons of mean performance of F1 progenies under free drainage condition and statistical significance for the difference between the progenies and their respective mid-parent values.

Crosses	DHE			DMA			SPL			NS/SP			GY/SP			GY/pot		
	F1	MP	t-test	F1	MP	t-test	F1	MP	t-test	F1	MP	t-test	F1	MP	t-test	F1	MP	t-test
P1 x P2[a]	84	91	**	141	145	NS	6.6	6.3	NS	42	36	NS	2.21	1.83	NS	9.15	8.34	NS
P1 x P3	87	90	NS	141	142	NS	6.7	6.4	NS	44	36	NS	2.01	1.88	NS	9.30	7.59	NS
P1 x P4	81	89	**	136	144	*	7.1	5.7	**	47	36	*	2.48	1.86	**	10.25	7.08	**
P1 x P5	88	91	NS	142	141	NS	7.6	6.7	**	41	41	NS	2.35	2.14	NS	10.82	8.55	*
P2 x P3	81	79	NS	137	135	NS	5.6	5.6	NS	33	28	NS	1.55	1.43	NS	8.65	8.04	NS
P2 x P4	78	78	NS	133	137	NS	6.2	4.9	***	31	28	NS	1.64	1.42	NS	7.61	7.53	NS
P2 x P5	81	81	NS	143	135	*	6.6	5.9	*	33	33	NS	1.65	1.69	NS	9.91	8.99	NS
P3 x P4	79	76	NS	132	134	NS	6.5	4.9	***	33	28	NS	1.64	1.46	NS	8.55	6.80	NS
P3 x P5	75	79	NS	130	132	NS	6.5	6.0	NS	31	32	NS	1.73	1.72	NS	8.14	8.27	NS
P4 x P5	79	78	NS	132	134	NS	6.2	5.3	**	43	33	*	1.97	2.00	NS	10.57	7.76	**

Table 8.14. Comparisons of mean performances of F1 progenies under waterlogged condition and statistical significance of the differences between the progenies and their respective mid-parent values.

Crosses	DHE			DMA			SPL			NS/SP			GY/SP			GY/pot		
	F1	MP	t-test	F1	MP	t-test	F1	MP	t-test	F1	MP	t-test	F1	MP	t-test	F1	MP	t-test
P1 x P2[a]	110	106	NS	144	155	***	7.4	6.3	***	34	30	NS	1.58	1.42	NS	5.29	5.54	NS
P1 x P3	104	104	NS	152	155	NS	7.0	6.2	*	41	27	*	1.87	1.36	NS	7.49	4.67	NS
P1 x P4	106	107	NS	151	154	NS	7.1	5.1	***	27	24	NS	1.54	1.08	NS	6.15	5.33	NS
P1 x P5	107	106	NS	153	155	NS	6.9	6.6	NS	29	26	NS	1.65	1.38	NS	7.61	4.79	NS
P2 x P3	92	93	NS	142	144	NS	6.6	6.1	NS	38	35	NS	2.02	1.74	NS	7.87	6.18	NS
P2 x P4	94	96	NS	142	143	NS	6.4	5.1	***	37	32	NS	1.63	1.46	NS	6.31	5.84	NS
P2 x P5	99	96	NS	139	144	NS	6.6	6.5	NS	29	34	NS	1.63	1.76	NS	6.49	6.30	NS
P3 x P4	101	94	NS	143	143	NS	7.1	5.0	**	40	29	NS	2.22	1.41	*	7.90	4.98	NS
P3 x P5	92	94	NS	138	145	*	6.8	6.5	NS	41	31	NS	2.10	1.71	NS	8.26	5.44	NS
P4 x P5	93	97	NS	139	144	NS	6.4	5.4	**	37	28	NS	2.09	1.43	NS	8.07	5.10	NS

P1=Feres Gama(37), P2=Feleme(68), P3=Mage-07, P4=1184(44), & P5=1153(28); *, **, & *** indicate significantly different values at 0.05, 0.01 & 0.001 probability levels, respectively. MP=Mid-parent value

8.4.5. Correlations among characters

The genetic correlation coefficients were generally higher than the phenotypic correlation coefficients in all cases. Moreover, the number of significant correlation coefficients was greater for the genetic than for the phenotypic correlation coefficients at both treatment levels (Tables 8.15 & 8.16). The number of significant genotypic correlations was more or less equal at both treatment levels but significant phenotypic correlations were less in waterlogged than free drainage conditions. The magnitude of differences of the two correlation coefficients was as low as 0.062 between days to heading and spike length to as high as 0.254 between days to maturity and grain yield per spike under free drainage conditions. In the waterlogged experiment, the two correlation coefficients were very close for days to heading vs spike length and days to maturity vs spike length. Genotypic correlations between grain yield and yield components (spike length, number of seeds spike^{-1} and grain yield spike^{-1}) were greater than one under waterlogged conditions. Since genetic correlation coefficients are functions of unobservable genotypic values, they are estimated only by indirect means and are difficult to measure with precision (Baker, 1986). Hence, though no two measurements can have a correlation greater than one, computationally, it is possible to have an estimate exceeding one (Baker, 1986) but the values shall be set to one if they are to be used for computing other parameters. Similar cases have also been reported (Rowe & Brink, 1993; Hebert et al., 1994) and results from this experiment are not exceptional.

In the free drainage experiment, days to heading and maturity had the lowest phenotypic and genotypic correlations with grain yield while the yield components had strong and positive association with grain yield both at the genotypic and phenotypic level. By contrast, in the waterlogged experiment, days to heading and maturity had very high and significant negative genotypic correlations with grain yield and very low but still negative phenotypic correlation. Moreover, days to heading and maturity had significant and negative genotypic correlations with number of seeds spike^{-1} and grain yield spike^{-1} under waterlogged conditions which is not the case in the free drainage experiment signifying the involvement of different genetic effects in determining the association between phonological characters and yield and yield components in the environments under consideration. The pattern of association (signs of correlations) between yield and yield

components was similar for both treatment levels, however. The negative genetic correlation coefficients that existed between days to heading and maturity with yield and yield components in the waterlogging experiment indicates that selection for one or both of these characters would result in the discarding of some of the desirable progenies for yield components if the selection pressure for the characters under consideration is very strong whereas the opposite is true in the free drainage experiment. The lack of significant genetic correlation of days to heading and maturity with grain yield, but significant positive genetic correlation with yield components under free drainage condition would allow the breeder to portray genotypes of different phenological groups, without exceeding the critical limits for heading and maturity, and at the same time selecting genotypes with desirable yield components. Differences in correlations between characters in stress and non-stress environments and inefficiency of selection in favourable environment for improving character in stress environments have been reported (Ceccarelli et al., 1992; Aastveit & Aastveit, 1993; Bouzerzour & Dekhili, 1995; Shakhatrel et al., 2001) which is also true in this case.

Table 8.15. Genotypic (G) and phenotypic (P) correlation coefficients of characters from a diallel cross of barley landrace lines evaluated under free drainage conditions in a greenhouse, 2002.

Character	Corr. type	DMA	SPL	NS/SP	GY/SP	GY
DHE	G	0.984**	0.616**	0.799**	0.805**	0.342
	P	0.849**	0.554*	0.598*	0.598*	0.182
DMA	G		0.545*	0.623**	0.689**	0.332
	P		0.387	0.390	0.435	0.101
SPL	G			0.853**	0.932**	0.853**
	P			0.707**	0.801**	0.660**
NS/SP	G				1.036**	0.820**
	P				0.959**	0.631**
GY/SP	G					0.744**
	P					0.634**

* P = 0.05

** P = 0.01

Table 8.16. Genotypic (G) and phenotypic (P) correlation coefficients of characters from a diallel cross of barley landrace lines evaluated under waterlogged condition in a greenhouse, 2002.

Character	Corr. type	DMA	SPL	NS/SP	GY/SP	GY
DHE	G	0.954**	0.378	-0.714**	-0.895**	-0.946**
	P	0.787**	0.312	-0.401	-0.494	-0.341
DMA	G		0.193	-0.652**	-0.932**	-0.851**
	P		0.166	-0.381	-0.536*	-0.320
SPL	G			0.595*	0.779**	1.129**
	P			0.348	0.448	0.425
NS/SP	G				0.934**	1.094**
	P				0.365	0.279
GY/SP	G					1.585**
	P					0.398

* P = 0.05
** P = 0.01

8.5. Conclusions

Genetic parameters for several characters were estimated based on diallel crosses of five barley landrace lines that differed in days to heading, days to maturity, spike length, seed colour and yield potential. The fact that there are two contrasting barley production environments in north Shewa (areas prone to waterlogging and areas free of waterlogging stress) and because there is evidence that genetic variances, genetic correlations between characters and effectiveness of selection differ in stress and non stress environments prompted the evaluation of the F1 progenies under conditions of free drainage and waterlogging stress. The results elucidated the importance of general combining ability (GCA) for days to heading and days to maturity at both treatment levels and for number of seeds spike^{-1} and grain yield spike^{-1} in the free drainage experiment only indicating the significance of additive gene effects for these characters. In free drainage conditions, grain yield appeared to be determined both by additive and dominant gene actions, however. Both GCA and SCA were significant for spike length at both treatment levels but the higher GCA:SCA ratio and the higher GCA variance (δ^2gca) in the free drainage experiment indicated that this character is also under the control of additive gene action. By contrast, although GCA:SCA ratio was higher for this character in the

waterlogging experiment, SCA variance (δ^2sca) was greater than the δ^2gca. Hence, spike length is not under the control of additive gene action under waterlogging stress.

The patterns of GCA effects of parents for days to heading and days to maturity are similar for both treatment levels but different for the other characters. Among the parents, the two late and early maturing lines, Feres Gama(37) and 1153(28), respectively contributed the highest positive GCA effects for yield and yield components in the free drainage experiment. Accordingly, the cross between these two parents gave the highest mean spike length, grain yield spike^{-1} and grain yield than all crosses and is among the top in number of seeds spike^{-1}. The fact that GCA effects of 1153(28) for number of seeds spike^{-1} and grain yield spike^{-1} were not significant imply that this parent is not as good a combiner as Feres Gama(37) for these characters, but it is as good as Feres Gama(37) in its combining ability for spike length. Moreover, the difference for GCA effects of the two parents for spike length is significant denoting that both parents are equally desirable. In conditions of waterlogging, Mage(07) had consistently higher positive GCA effects for yield and yield components than all other parents. The non significant GCA mean square values for yield and yield components except spike length under waterlogged conditions put the importance of this line in question, however.

GCA and SCA variance estimates associated with each parent also indicated that Feres Gama(37) and 1153(28) had comparable SCA variances for yield and yield components in the free drainage experiment suggesting that both parents transferred their genetic potential for yield and yield components effectively to their progeny. However, the relatively lower SCA variance for spike length associated with 1153(28) indicated that this parent transferred its genetic potential to its progeny better than Feres Gama(37). Under waterlogged conditions, neither yield nor yield components had significant GCA and SCA mean square values except spike length which was also the case in ordinary analyses of variance. Hence a comparison of GCA and SCA variances associated with each parent would not be fair. The negative estimates of genetic parameters observed for most characters under waterlogged conditions except for days to heading and maturity imply the difficulty in achieving the anticipated progress through selection for quantitative characters.

By the same token, the non significant GCA effects of parents under waterlogged conditions also illustrated the difficulty in predicting the performance of progenies under waterlogged conditions.

It can be generalized that Feres Gama(37) and 1153(28) were found to be good combiners and they shall be tested thoroughly in order that maximum use of their superior combining ability can be made in future crossing program aimed at improving yield and yield components for environments free of waterlogging problems. Grain yield spike^{-1} is highly heritable and predictable under free drainage conditions so that good progress can be expected from effective selection for this character. By contrast, the very low heritability (0.19) for grain yield implies that it is very difficult to make progress by selection for grain yield *per se*. Therefore, grain yield spike^{-1} can serve as indirect selection for genotypes with high grain yield since it has highly significant positive genotypic and phenotypic correlation with grain yield also. An important point to note is that the estimates of genetic variances and heritability values were from one experiment in one year. Therefore, the values are likely to be biased upward due to confounding effects of genotype x location, genotype x year and genotype x location x year interaction components. Nevertheless the estimates provide an indication of the relative ease of making progress through breeding.

CHAPTER IX

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Genetic variability is a key factor to success in any crop improvement program because the effectiveness of selection or amount of progress expected from selection is entirely dependent on genetic variability. On the other hand genetic variability has little value unless it is evaluated and utilized in the breeding program. In this research farmers' cultivars predominately grown in north Shewa, Ethiopia and landrace collections from environments where these cultivars are grown, were considered. The level of genetic variability within and between landraces and genetic relatedness between landraces was assessed at phenotypic, biochemical (SDS-PAGE of seed storage proteins) and molecular (AFLP) level so as to facilitate their use in a regional breeding program. Crossing between selected landrace lines differing in agronomic characters was done to predict the breeding potential of landraces and performance of their progenies. Genetic variability in response to waterlogging stress (to which landraces vary in adaptation) was also investigated.

Results from phenotypic variability study indicated that improvement in the economically important characters such as grain yield can be made if selection is exercised to isolate components of spike types in some of the farmers' cultivars (Bukura, Nech Ferke, Baleme and Haddo) and accessions showing variability for the character. Although the estimated diversity within landraces for the quantitative characters indicated greater variability, it would be difficult to explicitly conclude that progress can be made through selection because the variability manifested may not be solely due to genetic factors since environmental influence in the manifestation of quantitative characters is also great.

The level of genetic variability within and between landraces was investigated further with the help of SDS-PAGE of seed storage proteins to supplement results from morphological descriptors. The information obtained from SDS-PAGE was found to be helpful and complementary to morphological data in that decisions can be made on which landraces to focus for selection, multi-location testing and also make region specific crosses among the adapted landraces. Hence, in the utilization process of these

landraces, the first recommendation would be to separate landraces that visually appeared superior and at the same time were genetically heterogeneous, as revealed by SDS-PAGE, into their components derived from single spikes followed by performance evaluation in multi-locations to isolate the best lines. This will facilitate the evaluation and identification of best lines which otherwise would be difficult to distinguish genetically different and superior lines under field conditions because of environmental effects. Secondly, SDS-PAGE data would permit identification of genetically distant parental lines possessing desirable agronomic features so that long term crossing programs can be designed. The study demonstrated that divergence for morphological characters *per se* may not reflect the true genetic distance between landraces. Hence, selection of parents for crossing shall be supplemented based on variability for seed storage protein polypeptides to get the expected progeny variance.

Results from AFLP data clearly indicated that none of the farmers' cultivars were genetically identical. The fact that the accessions were not clustered together with the farmers' cultivars indicated that each cultivar may possess adaptive gene complexes unique to it that cannot be represented by random sampling of germplasm from areas of its production. Hence, a comprehensive study of a range of farmers' barley cultivars grown in different parts of Ethiopia is suggested in order to enhance their effective utilization either by the national or regional breeding programmess. The information from this study will also be helpful for the current *in situ* biodiversity conservation strategy being carried out by the Biodiversity Conservation and Research Institute in Ethiopia since it affirmed the distinctness of each cultivar from the other so that unnecessary exclusion of certain morphologically similar cultivars from conservation schemes can be avoided.

Field evaluation of pure lines from these farmers' cultivars showed lower genetic variability within, rather than between cultivars and this was further verified by SDS-PAGE of seed storage proteins. Hence, genetic gain through selection for yield and yield components within the farmers' cultivars from this particular environment is expected to be minimal. Therefore, carefully removing the inferior types to upgrade yield potential is suggested as an easy and short cut strategy rather than attempting to improve them through extensive pure line selection and evaluation methods. Evaluation of landraces from other sources, from within or outside the environment, is suggested to

get better yielding landraces than the existing ones. In the long term, crossing shall be scheduled between cultivars or accessions which, based on morphological characters and protein profiles appear to be diverse in order to maximize the potential gain from selection in the progeny.

Evaluation of F1 progenies from a 5 x 5 diallel crosses of five barley landrace lines [Feres Gama(37), Feleme(68), Mage(07), 1182(44) and 1153(28)] that differed in days to heading, days to maturity, spike length, seed colour and yield potential demonstrated possibility of identifying superior parents from locally adapted landraces that can allow selection of superior recombinant lines. The success of identifying the superior recombinant lines will be highly dependent on where the progenies are to be evaluated (waterlogged or free drainage conditions), however. This was because of differences observed in the estimates of genetic parameters and combining ability of parents when the crosses were evaluated under the fore mentioned environmental conditions. Fore instance, under free drainage conditions Feres Gama(37) and 1153(28) contributed the highest GCA effects for yield and yield components which was not the case under waterlogged conditions. Moreover, negative estimates of genetic parameters and non significant GCA effects of parents under waterlogged conditions indicated the difficulty in capturing the variability because of large environmental noise which was not observed under free drainage conditions. This indicates that, under conditions of waterlogging, one may need to consider more sophisticated experimental designs and analysis (fore example spatial analysis) when evaluating breeding materials under field conditions in order to capture the variability within the progenies.

Three of the five parents used in a diallel analyses [Feres Gama(37), Mage(07) and Feleme(68)] were studied for their variability in growth and nutrient uptake under waterlogging stress. It can be concluded that differences between the susceptible (Feres Gama(37) and tolerant (Mage(07) landraces in response to waterlogging were largely due to less dry matter accumulation of the tillers and a reduced top growth in the susceptible landrace more than for the tolerant landrace. Moreover, differences between the tolerant and susceptible landraces were due to P concentration and uptake the effect being less on the tolerant landrace than on the susceptible landrace Feres Gama(37). Difference in N concentration of shoots between Feres Gama(37) and Mage(07) was also observed although the magnitude was not comparable to that of P. Hence, genetic

differences between landraces in response to waterlogging comply with the farmers' opinion.

To coin, findings from the different set of experiments highlighted that barley breeders in regional centres should seek more to make use of landraces adapted to specific environmental conditions in order to achieve specific goals pertaining to their target environment.. The national breeding programme should also put more effort to the realization of decentralized breeding programme. Crosses, and the resulting pre-national and national variety trials should be set depending on the target environments so that the regional and national programmes proceed in complimentary in order to achieve the pre-set objectives of either the national or regional programmes.

CHAPTER X
SUMMARY

Genetic variability and breeding potential of barley landraces from north Shewa in Ethiopia was studied with the aims i) to generate information about the level of diversity within and among the landraces in terms of morphological descriptors, SDS-PAGE of seed storage proteins, and Amplified Fragment Length Polymorphism (AFLP) ii) to assess yield relationships and expected genetic advance that may be achieved through selection within or among landraces iii) to estimate genetic variances for agronomic characters from crosses involving selected barley landrace lines and predict the breeding potential of landraces and iv) to prove genetic differences between landraces, claimed by farmers, in response to waterlogging stress. The phenotypic diversity of 44 landraces, including farmers' cultivars, was estimated using Shannon Weaver diversity index for different qualitative and quantitative characters. This was further supplemented with SDS-PAGE of seed storage proteins and the two measures of variability were compared. Of the 44 landraces, emphasis was given to farmers' cultivars from which five to six pure line derivations from them were evaluated under field conditions to assess potential differences for agronomic characters and to estimate genetic advance that may be achieved through selection within the cultivars. Variability between lines within the cultivars for the quantitative characters was further assessed with the help of SDS-PAGE. Diallel crossing between five selected landrace lines differing in agronomic characters was carried out and genetic parameters and breeding potential of the parents were estimated. Three of the parents used for crossing were further investigated for their differences in growth and nutrient accumulation under waterlogging stress in a greenhouse pot experiment.

Both morphological and SDS-PAGE data demonstrated the variability existing in the landraces. $\overline{H'}$ values pooled over morphological characters ranged from 0.12 ± 0.08 to 0.57 ± 0.11. Among the qualitative characters, landraces showed higher levels of polymorphism for spike type than for kernel colour, 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. 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 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.

AFLP analysis of the genetic relationships between farmers' cultivars using six primer combinations illustrated varying degrees of genetic dissimilarity (0.372 to 0.728) with a mean of 0.545. Thirty one percent of the values in this range were $\leq$ 0.500 while 69 % of the values were $\geq$ 0.600 demonstrating the presence of sufficient variation between the farmers' cultivars. The number of scoreable fragments amplified by each AFLP primer combination varied from 49 to 118 with an average of 84.5 and polymorphic fragments for each primer combination varied from 27 to 77 the average being 58.5. The average percent polymorphism was 69.9 % with values ranging from 55.1 % to 75.8 %.

Field evaluation of pure lines from farmers' cultivars indicated that genetic gain through selection for yield and yield components within the farmers' cultivars from this particular environment is minimal. Selection may even end up with negative gain unless carefully carried out to isolate those that might be useful to bring slight improvement in yield. Carefully excluding the inferior types to upgrade yield potential is suggested rather than attempting to improve them through extensive pure line selection and evaluation methods. Evaluation of landraces from other sources, from within or outside this parrticular environment, is suggested to get better yielding landraces than the existing ones. In the long term, crossing may be scheduled between cultivars which, based on morphological characters and protein profiles, appear to be diverse in order to maximize the potential gain from selection in the progeny.

Estimated genetic parameters from diallel crosses of barley landrace lines highlighted the importance of additive gene actions for spike length, number of seeds $spike^{-1}$ and grain yield $spike^{-1}$ under free drainage conditions and for days to heading and days to maturity at both treatment levels. Both additive and non-additive gene actions were important in the control of grain yield under free drainage conditions. By contrast, estimates of genetic parameters for yield and yield components (except spike length) were very low or negative under waterlogged conditions. Among the parents, Feres Gama(37) and 1153(28) contributed the highest positive GCA effects and comparable SCA variances for yield and yield components under free drainage conditions. Hence, these parents shall be tested thoroughly in order that maximum use of their superior combining ability can be made in future crossing programs for environments free of waterlogging stress. A separate crossing and selection program is suggested for the respective environmental conditions if resources permit.

Waterlogging reduced the total number of tillers per pot of the tolerant landrace Mage(07) and Feleme(68) by 43 % and 48 %, respectively compared to the control plants while it was only 23 % for the susceptible landrace Feres Gama(37). Total shoot dry matter accumulation under waterlogged conditions was comparable for all three landraces in spite of the largest number of tillers produced by Feres Gama(37). Differences between the susceptible and tolerant landraces in response to waterlogging were largely due to less dry matter accumulation of the tillers and slower growth in the susceptible landraces. Moreover, apparent differences were noticed in P concentration and uptake between the tolerant and susceptible landraces, the effect being less for the tolerant landrace Mage(07) than for the susceptible landrace Feres Gama(37). Difference in N concentration of shoots between Feres Gama(37) and Mage(07) was also observed although the magnitude was not comparable to that of P.

OPSOMMING

Genetiese variabilitieit en teel potensiaal van gars landrasse van noord Shewa in Etiopië is bestudeer met die doel om (i) inligting te genereer oor die vlak van diversiteit binne en tussen die landrasse in terme van morfologiese beskrywers, SDS PAGE van saad storings proteïene, en AFLP (Amplified Fragment Length Polymorphism) (ii) om opbrengs verwantskappe en verwagte genetiese vordering wat gekry kan word deur seleksie te bepaal tussen en binne landrasse (iii) om genetiese variansies te bepaal vir agronomiese eienskappe wat kruisings insluit van geselekteerde gars landras lyne en om die teel potensiaal van landrasse te bepaal (iv) om genetiese verskille tussen landrasse wat deur boere gerapporteer is te bevestig vir reaksie op versuipings stremming. Die fenotipiese diversiteit van 44 landrasse, insluitend boere cultivars, is bepaal met die Shannon Weaver diversiteits indeks vir verskillende kwantitatiewe en kwalitatiewe eienskappe. Dit is verder aangevul met SDS-PAGE van saad storings proteïene en die twee metings van variabiliteit is vergelyk. Van die 44 landrasse, is klem geplaas op die boere cultivars waarvan vyf tot ses ontwikkelde suiwer lyne geëvalueer is onder veld toestande om potensiële verskille in agronomiese eienskappe te bepaal en om genetiese vooruitgang te bepaal wat gemaak kan word deur seleksie binne hierdie cultivars. Variabiliteit tussen lyne binne cultivars vir die kwantitatiewe eienskappe is verder geëvalueer met die hulp van SDS-PAGE. 'n Dialleel kruising tussen vyf geselekteerde landras lyne met verskillende agronomiese eienskappe is gedoen, en genetiese parameters en telings potensiaal van die ouers is vasgestel. Drie van die ouers wat gebruik is in kruisings is verder ondersoek vir verskille in groei en voedingstof akkumulsie onder versuipings stremming in 'n glashuis pot proef.

Beide morfologiese en SDS-PAGE data het getoon dat variabiliteit bestaan in die landrasse. H' waardes wat gepoel is oor morfologiese eienskappe het gewissel van 0.12±0.08 tot 0.58±0.11. Binne kwalitatiewe eienskappe het landrasse hoër vlakke van polimorfisme getoon vir aar tipe as vir korrel kleur, aar kompaksie en kariopsis tipe (bedek of nakend). Kariopsis tipe was die minste diverse eienskap wat gesien is. Diversiteit vir kwantitatiewe eienskappe gepoel oor landrasse was oor die algemeen baie hoog, veral vir saad per aar en dae tot volwassenheid met H' waardes van 0.90 en 0.98. SDS-PAGE data, gebasseer op verteenwoordigende lyne van elke landras het baie

lae tot hoë binne landras variabiliteit getoon. Lyne van landrasse het verskil van mekaar in aantal en migrasie afstande van bande. Sommige landrasse wat univorm gelyk het vir aar morfologie het verskille in bandpatrone getoon. Daar is ook gesien dat sekere landrasse wat verskillende aar eienskappe toon, en waar storings proteïen band verskille verwag is, het nie veel verskille getoon nie. Variabiliteit tussen landrasse was hoër as binne landrasse, en variabiliteit binne boere cultivars was laer as binne ander inskrywings. Groeperings resultate van SDS-PAGE data was verskillend in samestelling van die van morfologiese eienskappe. Groepering van morfologiese data het definitiewe groepe landrasse gegee gebasseer op ooreenkomste in morfologiese eienskappe, terwyl SDS-PAGE nie dieselfde gewys het nie.

AFLP analise van genetiese verwantskappe tussen boere cultivars met ses priemstuk kombinasies het verskillende vlakke van genetiese verskille (0.372 tot 0.728) aangetoon met 'n gemiddeld van 0.545. Een en dertig persent van die waardes in hierdie reeks was ≤ 0.500 terwyl 69% van waardes ≥ 0.600 wat die teenwoordigheid van genoeg variasie bewys tussen boere cultivars. Die aantal bruikbare fragmente geamplifiseer deur elke AFLP priemstuk kombinasie het gevarieër van 49 tot 118 met 'n gemiddeld van 84.5 en polimorfiese fragmente van elke priemstuk kombinasie het gewissel van 27 tot 77, met 'n gemiddeld van 58.5. Die gemiddelde persentasie polimorfisme was 69.9% met waardes tussen 55.1% tot 75.8%. Veld evaluasie van suiwer lyne van boere cultivars het getoon dat genetiese vooruitgang deur seleksie vir opbreangs en opbrengs komponente binne boere cultivars vir hierdie omgewing minimaal is. Seleksie kan selfs negatiewe vordering toon tensy dit versigtig gedoen word om daardie eienskappe te isoleer wat nuttig kan wees om 'n effense verbetering in opbrengs te gee. Versigtige uitsluiting van swak tipes om opbrengs potensiaal te verhoog is beter as intensiewe suiwer lyn seleksie en evaluasie metodes. Evaluasie van landrasse van ander bronne, van binne of buite die gebied is voorgestel om landrasse te kry wat beter opbrengs gee as die huidiges. In die lang termyn kan kruisings gemaak word tussen cultivars wat, gebasseer op morfologiese eienskappe en proteïen profiele, divers is sodat maksimum potensiaal vooruitgang gekry kan word van seleksie uit die nageslagte.

Genetiese parameters vanaf dialleel kruisings van gars landrasse het die belangrikheid van additiewe geen aksie vir aar lengte, aantal sade per aar en graan opbrengs per aar

onder vry dreinasie toestande en dae tot aarstoot en dae tot volwassenheid by beide behandelings, aangetoon. Beide additiewe en nie-additiewe geen aksies was belangrik in die beheer van graan opbrengs onder vrye dreinasie toestande. In teenstelling hiermee, was bepalings van genetiese parameters vir opbrengs en opbrengs komponente (behalwe vir aar lengte) baie laag of negatief onder versuipings toestande. Tussen die ouers, het Feres Gama(37) en 1153(28) die hoogste positiewe GCA effekte en vergelykbare SCA variansies vir opbrengs en opbrengs komponente onder vrye dreinasie gehad. Daarom sal hierdie ouers deeglik getoets word om maksimum gebruik van hulle goeie kombineervermoë te maak in toekomstige kruisings programme vir omgewings vry van versuipings stremming. 'n Aparte kruisings en seleksie program vir verskillende omgewings toestande word aanbeveel as hulpbronne dit toelaat.

Versuiping het die aantal stamme per pot by die tolerante landrasse Mage(07) en Feleme(68) met 43% en 47% afsonderlik verminder in vergelyking met die kontrole plante maar vir die vatbaar landras Feres Gama(37) was dit net 23%. Totale stam droë materiaal akkumulasie onder versuipings toestande was vergelykbaar vir al drie die landrasse al het Feres Gama(37) die meeste stamme geproduseer. Verskille tussen vatbare en tolerante landrasse in reaksie op versuiping was grootliks a.g.v. minder droë materiaal in die stamme en stadiger groei in die vatbare landrasse. Verder was daar duidelike verskille in P konsentrasie en opname tussen tolerante en vatbare landrasse, waar die effek minder was vir tolerante landras Mage(07) as vatbare landras Feres Gama(37). Verskille in N konsentrasie van stamme tussen Feres Gama(37) en Mage(07) was ook duidelik, maar die verskille was kleiner as vir P.

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