GENETIC DIVERSITY ANALYSIS OF LINSEED (*LINUM USITATISSIMUM* L.) IN DIFFERENT ENVIRONMENTS

By

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DECLARATION

I declare that the thesis hereby submitted by me for the degree of Philosophiae Doctor in Agriculture at the University of the Free State is my own independent work and has not previously been submitted by me to another University/ Faculty.

I further cede copy right of the thesis in favour of the University of the Free State.

Adugna Wakjira Gemelal November 29, 2002

DEDICATION

This piece of work is dedicated to my father Wakjira Gemelal, my mother Damashi Erenssa and my elder sister Qanani Wakjira who provided me with the opportunity of education and remained on their farms, appreciating the fruits of agricultural diversity and challenges.

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CHAPTER 1

GENERAL INTRODUCTION

Genetic diversity can be expressed through the very large number of associations or combinations of genes which exist in the individuals of a single species and are shown as characters that differ among the cultivated varieties of the same plant species in growth pattern, resistance to pests, tolerance to environmental conditions (frost, heat, drought, etc.) and productivity (Frankel and Brown, 1984; Haque *et al.*, 1994). Genetic diversity, which is the heritable portion of observable variation (Kresovich and McFerson, 1992), is a crucial ingredient in the crossbreeding or hybridisation processes aimed at giving more vigour (McNaught, 1988) to the crop varieties that have been cultivated over the last thousand years. It is also a safety factor against climatic stress and pests. Genetic diversity offers the guarantee of achieving a reasonably good harvest and this explains why farmers grow several varieties of crops (McNaught, 1988), which differ in their agronomic traits and their resistance to climatic and disease stresses.

Efficient identification and selection of desirable genotypes largely depend on a comprehensive understanding of the genetic relatedness and variation existing within the crop and its closely related wild species (Kearsey, 1993; Kresovich and McFerson, 1992). Information concerning genetic relatedness is crucial for it indicates the rate of adaptive evolution and the extent of response in crop improvement (Vega, 1993). Furthermore, it is essential as a guideline in the choice of parents for breeding programs (McNaught, 1988), to detect genetic duplicates in germplasm collections and implement an effective genetic conservation program (Frankel and Brown, 1984).

Morphological grouping of linseed is based on characters such as yield, seed colour, earliness, plant height, growth habit, flower colours and disease reactions (Seegler, 1983). Unfortunately, these characteristics are a result of interactions of genes and their products, and the environment in which they are grown (Russell, 1986; Tanksley, 1983). Furthermore, traits of agronomic interest like vigour,

disease resistance and cold tolerance usually involve high genotype-environment interactions. As a result, there are limited numbers of stable traits that can be used to distinguish differences. Moreover, the requirement of several months to observe the distinguishing characteristics (Vega, 1993), subjectivity included in observation, difficulty of discriminating closely related genotypes and species due to a low level of polymorphism (Kumar, 1999) makes this method problematic. Hence, morphological and agronomic evaluation of population variability needs to be supplemented through direct study of the genome.

Diversity can be measured as the number of different organisms and their relative frequency at genus, species, population, individual, genome, locus and DNA base sequence (Kresovich and McFerson, 1992; Gaston, 1998; Kumar, 1999). However, the process of measurement needs to be iterative and dynamic because micro- and macro evolutionary changes will occur everywhere (Gaston, 1998). Similarly, cost-effective detection of variation, employing the appropriate tool is the key in the assessment processes for genetic representation and accession uniqueness based on genetic distance. This becomes even more important in the future as concern for property rights of plant genetic resources increases nationally and globally (Kresovich and McFerson, 1992). Hence, genetic divergence plays a vital role in the construction of a successful breeding program. The genetically diverse parents are likely to produce the high heterotic effects and the yield desirable segregates. Thus quantitative assessment of genetic divergence is necessary to decide the nature and extent of genetic differences among crop species.

Linseed (*Linum usitatissimum* L.) is a diploid annual field crop that has been largely grown in temperate climates including the highlands (>2500 meters above sea level) of Ethiopia. It is the second most important oil crop of Ethiopia in terms of area and production (Adugna, 2000). Linseed has been cultivated in Ethiopia since antiquity (Adefris *et al.*, 1992). In 1996 alone, it was grown on about 148 000 hectares with a production of about 68 000 tons and with a productivity of about 0.46 t/ha (CSA, 1997). The main linseed producing areas of Ethiopia are the southeastern regions of Arisi, Bale, eastern Wellega, eastern Gojam, Semen mountains, Tigray, western Wello and highlands of Hararghe and Shewa (Adugna, 2000). The principal regions of linseed production have an altitude range of 1200 to 3500 meters above sea level

and the crop performs best within 2200-2800 m. Linseed requires cool temperatures during its growing period to produce good yields. The mean temperature can range from 10°C to 30°C although it does best from 21-22° C. The crop grows well within a 12 to 18 hour photoperiod (Adugna, 2000).

Linseed has been cultivated in Ethiopia for two primary purposes, seed and oil yields. It has traditionally been used for food and as a cash crop since ancient time (Seegler, 1983). Linseed oil has many food and industrial applications (Rowland, 1990), and its seed cake is also a valuable feed for livestock (Pizzey, 1998). Linseed oil has 55-58% of unique alfa-linolenic fatty acid (an essential omega 3 fatty acid), which has beneficial effects on health and the auto immune system (Carter, 1993; Bhatty, 1995; Aldrich, 1998). Alpha-linolenic was reported (Carter, 1993; Flax Council of Canada, 2000a; Payne, 2000) to have the effects of anti-hypercholestrolemic, anti-carcinogenic and important for the normal developments of brain and retinal tissues of infants. The soluble fibre of linseed helps to lower blood cholesterol, while insoluble fibre promotes laxative effects (Payne, 2000). Moreover, its lignan (phytoestrogen, plant compound with estrogen-like activity) are found useful for women's health (Flax Council of Canada, 2000b; Payne, 2000). As the result, linseed is currently well recognized for its functional food products.

Preliminary collection and characterization of linseed has been underway in Ethiopia at Holetta Research Centre since the early 1980s (Getinet *et al.*, 1987) in collaboration with the Institute of Biodiversity Conservation and Research (IBCR). As the result, 641 collections were available in the IBCR (Abebe *et al.*, 1992). In 1981, 130 accessions were collected from eight administrative regions of Ethiopia (Getinet *et al.*, 1987). A year later, 129 accessions were characterized at Holetta for 15 traits. Only descriptive statistics (range, mean, standard deviation and frequency distribution of major traits) were employed to analyse their data. These characterization activities were kept on to select and advance elite materials, and also to rejuvenate the germplasm collections. However, systematic and in depth studies were not conducted to generate ample information that is required by the current and future breeding programs. But to make use of important genes, genetic diversity and eco-geographic pattern of variability should be studied and these are not yet done for linseed accessions in Ethiopia. Nevertheless, Ethiopia is the centre of diversity for

linseed and the crop has been one of the most important oil crops in the country (Seegler, 1983; Adugna and Adefris, 1995). Moreover, a wide range of agro-climatic conditions prevailing in the country (Adugna, 2000) may continue to contribute to the diversity of this crop. Thus the existing diversity could be exploited for the current and future breeding programs. In short, information on genetic diversity of linseed is very meagre and this study was undertaken to address these issues.

Proper characterization and evaluation of germplasm collections are important components for effective management of genetic resources and their utilization in the breeding programs (Frankel, 1989). Accurate identification of genotypes or varieties is very useful throughout the processes of breeding, starting from initial parent selection to the final utilization of cultivars in production schemes. Morphological or phenotypic descriptors have traditionally been used to distinguish one accession from the other. Although these types of agronomical characterization provide useful information to the users, they are subjected to environmental influences, time-consuming and they must be assessed during a fixed vegetative phase of the crop. Conversely, the biochemical methods such as storage proteins and DNA markers are accurate detectors, independent from the environment and the crop growing cycle (Kumar, 1999). However, they require specialist knowledge, laboratory equipment and chemical supplies that make them more expensive than the morphological descriptors. Therefore, using both morphological and biochemical characterizations can provide complementary advantages.

Modern techniques such as Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeats (SSR) DNA markers have proved to be efficient and reliable in supporting conventional plant breeding programs (Paterson *et al.*, 1991; Kumar, 1999). Marker-assisted breeding or selection has been offering the potential of deploying favorable gene combinations and for predicting better outcomes. An AFLP genetic linkage map of linseed was used to identify two quantitative trait loci (QTL) on independent linkage groups with a major effect on resistance to Fusarium wilt, a deadly disease of linseed (Spielmeyer *et al.*, 1998). These workers illustrated the potential of AFLP as a powerful and fast method to generate moderately saturated linkage maps, allowing molecular analysis of traits

like Fusarium wilt, that show oligogenic patterns of inheritance. Likewise, Inter-Simple Sequence Repeat amplification (ISSR) and Random Amplified Polymorphism DNA (RAPD) were used to elucidate the origin and segregating patterns of microspore-derived plants in anther culture of linseed (Chen *et al.*, 1998).

Moreover, Hausner and his co-workers (1998) have developed the co-dominant PCR/RFLP based markers for the flax rust resistance alleles. Their result confirmed that L^6 was present in many previously released cultivars, while L^9 was detected in recently released Canadian cultvars. All of these evidences indicated that molecular markers are useful in marker assisted selection (MAS) and in introduction of new genes for resistance of diseases like rust, Fusarium wilt and other vital traits in linseed breeding programs. AFLP markers were found as a robust and rapid method to generate moderately saturated linkage maps in linseed, enabling molecular analysis of traits like Fusarium wilt tolerance (Spielmeyer *et al.*, 1998). The AFPLs are used to draw dendograms to assess genetic relationships between the entries. This will, therefore, allow breeders to make a more informed choice of breeding parents.

Hypothesis and objectives of the study

The current linseed improvement program in Ethiopia, which is geared towards developing high yielding, good quality and disease tolerant varieties, has a good linkage with the University of Saskatchewan in Canada. Subsequently, some tissue culture derived regenerants variety were introduced to Ethiopia since early 1990. Moreover, there were many earlier introductions and several local collections that need to be utilised effectively in the breeding programs. For proper utilisation, however, the available germplasm has to be thoroughly analysed both morphologically and biochemically, employing more reliable tools. The development of comprehensive, well documented and accessible germplasm and willingness of investing resources in the long-term programs of germplasm diversification need to be the essential purposes of plant breeding. Identifying and manipulating the appropriate varieties can improve low yield and poor oil quality of linseed varieties. However, this was not sufficiently studied and little information is available on the accessions of Ethiopian linseed. Fatty acid profiles

have not yet been studied to meet the various needs of growers, consumers and traders of linseed for both food and industrial uses. Moreover, DNA markers are not yet employed in discriminating the various accessions of linseed in Ethiopia. The hypothesis is, therefore, there could be tremendous genetic diversity among the accessions of linseed collected from Ethiopia and some introductions, as well. In this context, the overall objective of this study was to analyse and describe the magnitude of genetic diversity in the Ethiopian linseed accessions (representative samples) for the benefits of future breeding programs, and the specific objectives were as follows:

1. To study the genetic diversity of 60 Ethiopian linseed accessions under glasshouse and field conditions, and to estimate phenotypic and genotypic coefficients of variation, heritability and genetic advance of useful traits.

2. To analyse oil contents and fatty acid profiles, determine the extent of genetic diversity and identify genotypes with desirable oil qualities for consumption and industrial uses.

3. To estimate the magnitude of genetic diversity using AFLP markers and classify the accessions in different groups based on their genetic distances.

4. To investigate and compare the relative advantages of both morphological descriptors and the AFLP markers for their usefulness in discriminating the accessions.

5. To determine the extent of relationships among the oil content, fatty acid composition, and other important characters.

6. To identify divergent and important accessions for the future breeding programs that could contribute to productivity of linseed.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Plant collection has enhanced agro-biodiversity since ancient time, and the earliest record goes back to 1495 B.C., when Egyptians gathered plants for food, medicine and other purposes from various parts of the world (Reid and Miller, 1989). Similarly, Mesoptamians, Chinese and Andean civilizations and their ancient agricultural settlements made use of diverse plants and agro-ecosystems (Vavilov, 1949; 1951; Reid and Miller, 1989). Throughout the colonial period, the search for and collection of diverse plants and foods was a driving interest of European explorers and played an important role in colonial expansion (Jones, 1983). Starting in the late 19^{th} and early 20^{th} centuries, scientists who recognized the value of diverse crop varieties discovered plant breeding methods that have boosted the productivity of our major crops.

Also significant was the work of N.I. Vavilov, a famous Russian botanist who carried out systematic plant collection, pioneering research, and conservation of crop diversity starting in the early 20th century (Reid and Miller, 1989; Eigenbrode, 1996). Vavilov developed a theory of the origin of domesticated crops and launched numerous worldwide expeditions to collect crop germplasm. He established an immense seed bank in St. Petersburg that still endures; now containing some 380000 specimens from more than 180 locations in the world (Reid and Miller, 1989). Vavilov also identified major areas of high concentrations of crop diversity around the world, most of which are in the developing countries (Vavilov, 1951). In other words, the need to conserve crop germplasm has been recognized since the work of Vavilov. He explored large areas of the world from 1920 to 1943, and made an inventory of cultivated plant species, their primitive and wild relatives (Reid and Miller, 1989). After a comprehensive evaluation of collections, he identified a concentrated diversity of certain crop species in some regions of the world, which were mostly separated by mountains, prairies and deserts. These regions were located in the northern temperate climatic zones between 20 and 45

degrees of latitude; however, later on the lowlands were proven to be among the centers of diversity (Kuckuck *et al.*, 1991). They were designated as gene centers or centers of origin of crop plants.

Vavilov summarized the results of his extensive research work in his theory of gene centers (Vavilov, 1951), where it condensed the knowledge of the 1920s and 1930s on the origin, descent and diversity of crop plants, interpreted and put it in logical context. It also stimulated in a unique way all branches of crop research, from taxonomy and evolution to plant physiology and biochemistry (Reid and Miller, 1989). Likewise, it accelerated the transformation of plant breeding from an empirical to a scientific basis (Kuckuck *et al.*, 1991). It also encouraged the establishment of worldwide collections and thorough studies of the huge wealth of genetic resources of our crop species and their relatives. Consequently, an increasing number of germplasm collection expeditions were mobilized all over the world to safeguard and utilize our valuable plant genetic resources (Kuckuck *et al.*, 1991), in order to combat genetic erosions and/or disasters, focusing on the geographical patterns of crop variation (Harlan, 1975; Rush, 1991). Still, accelerated programs are required to acquire, maintain and evaluate for use, as wide as possible a range of genetic diversity of crop plants, before they are lost forever because of man's adverse effects on natural environment and changes being made in agricultural patterns and practices (Jones, 1983; Esquinas-Alcazar, 2002). Nevertheless, to be of use to the breeder, the germplasm have to possess useful alleles, and these alleles can be identified through appropriate evaluation and/or analysis methods. Such studies will not only increase the values of the existing genetic resources but also help them to remain as a solid foundation for future development of agriculture in a sustainable manner.

In this literature review, therefore, efforts were done to disclose the main sources of information on the genetic diversity of crops in general and that of linseed in particular. More relevant topics in the areas of crops genetic diversity were generally presented to consolidate our understanding on this subject. The rationale for studying genetic diversity of crops and their values in the plant breeding programs were described along with the characterization and evaluation processes. The various approaches of diversity analyses and measurements were highlighted

together with their merits and demerits. The existing limitations in using genetic resources, the major causes for the ever-declining genetic resources and possible solutions to mitigate the problems were also discussed. The main theme of the study, genetic divergence of linseed and its associated points were also reviewed within the context of available literature. In short, attempts were made to highlight both the conventional and modern techniques of diversity analysis for better understanding that could assist the future improvement efforts.

2.2 Definition of genetic diversity and related terms

Biological diversity or biodiversity is defined as the total diversity and variability of living things and of the systems of which they are a part (Biodiversity Editorial Committee, 1996; Gaston, 1998). This covers the total range of variation in and variability among systems and organisms, at the bioregional, landscape, ecosystem and habitat levels, at the various levels of organisms, species, populations, individuals and genes. It also covers the complex set of structural and functional relationships within and between these different levels of organization, and their origins and evolution in space and time (Kresovich and McFerson, 1992). In other words, biodiversity refers to the number and variety of living organisms on earth, the millions of plants, animals, and micro-organisms, the genes they contain, the evolutionary history and potential they encompass, and the ecosystems, ecological processes, and landscapes of which they are integral parts. Biodiversity thus refers to the life-support systems and natural resources upon which we depend. In other words, it makes it possible to increase the number of foodstuffs available. Biodiversity has three main components of genetic diversity, species diversity and ecosystem diversity, as described below.

Genetic diversity: refers to the variation of genes within species, making it possible to develop new breeds of crop plants and domestic animals, and allowing species in the wild to adapt to changing conditions. In other terms, genetic diversity denotes the variability in the genetic characteristics of organisms that could belong to the same or different classification levels. In crop plants, genetic diversity arises as a consequence of interplay of evolutionary forces (mutation, selection and random drift) and the influence of man through domestication and selection (Allard, 1988). Genes are the biochemical packages which are passed on by parents to their

offspring, and which determine the physical and biochemical characteristics of offspring.

Species diversity: refers to the variety and abundance of species within a geographic area. A species is a group of plants or animals whose genes are so similar that they can breed together and produce fertile offspring. Usually different species look different. Species richness refers to the number of different species within a region. Jones (1983) emphasized that seeds of different crop species have in-built capacity for change. Random segregation of chromosomes to the spores in meiosis and their random recombination in the zygote at fertilization provide for genetic diversity among the offspring of the species. It is this genetic diversity that permits species to survive the biological and environmental stresses that vary within normal limits.

Ecosystem diversity: can refer to the variety of ecosystems found within a certain political or geographical boundary, or to the variety of species within different ecosystems (Biodiversity Editorial Committee, 1996). An ecosystem consists of communities of plants and animals and the soil, water, and air on which they depend. These all interact in a complex way, contributing to processes on which all life depends such as the water cycle, energy flow, the provision of oxygen, soil formation and nutrient cycling (Gaston, 1998). Similarly, plant **genetic resource** or **germplasm** comprises of the whole genetic heritage of all varieties of plants cultivated in any given region, as well as its wild and semi-domesticated relatives. The larger this genetic heritage is, the wider the biological diversity and the greater the potential it offers for the improvement of cultivated plant varieties or for the selection of new cultivars.

2.3 Rationale for studying genetic diversity of crops

In agriculture, genetic diversity can enhance production, as several varieties can be planted in the same field to minimize crop failure, and new varieties can be bred to maximize production or adapt to adverse or changing conditions (Clawson, 1985; McNaught, 1988). Genetic diversity is not only important in increasing yields, but also in maintaining the existing productivity (Altieri, 1987). For example, introducing genetic resistance to certain insect pests can increase crop yields, but

since natural selection often helps insects quickly overcome this resistance, new genetic resistance has to be periodically introduced into the crop just to sustain the higher productivity. Pesticides are also overcome by evolution of pests, so another important agricultural use of genetic diversity has been to offset productivity losses from pesticide resistance. Indeed, the record (Allard, 1990) shows that pesticides only temporarily conquer pests.

Biodiversity and its detailed knowledge have allowed farming systems to evolve since agriculture began some 12000 years ago (Reid and Miller, 1989). Agro-biodiversity is a fundamental feature of farming systems around the world. It encompasses many types of biological resources tied to agriculture. Agro-biodiversity therefore includes not only a wide variety of species, but also the many ways in which farmers can exploit biological diversity to produce and manage crops, land, water, insects, and biota. The concept also includes habitats and species outside of farming systems that benefit agriculture and enhance ecosystem functions (Altieri, 1987). The components of agro-biodiversity yield an array of benefits. They reduce risk and contribute to resilience, food security, and income generation. They also improve the health of soils.

In general, experience and research have shown (Chang, 1977; Altieri, 1985; Altieri and Anderson, 1986; Altieri, 1987; Reid and Miller, 1989) that agro-biodiversity can:

- Increase productivity, food security, and economic returns;
- Reduce the pressure of agriculture on fragile areas, forests, and endangered species;
- Make farming systems more stable, robust, and sustainable;
- Contribute to sound insect pest and disease management;
- Conserve soil and increase natural soil fertility and health;
- Contribute to sustainable intensification;
- Diversify products and income opportunities;
- Reduce or spread risks to individuals and nations;
- Help maximize effective use of resources and the environment;
- Reduce dependency on external inputs;
- Improve human nutrition and provide sources of medicines and vitamins; and

- Conserve ecosystem structure and stability of species diversity.

The importance of plant genetic resources or germplasm in the improvement of cultivated plants has been well recognized (Harlan, 1975; 1992; Frankel and Brown, 1984). Crop breeders in any country are primarily interested in utilizing the available germplasm for achieving specific breeding objectives. Hence, the required germplasm should consist of different types of the genetic variability for yield; yield components, plant height, maturity, resistance to diseases and pests and other stress conditions, and quality. Crop breeders would thus like to diversify the sources of useful variability for specific traits in germplasm collection to meet future requirements. The available stock of germplasm collections could possess a number of duplications for various traits. Subsequently, a lot of resources could be unnecessarily devoted to maintain them. Hence, effective and efficient ways of diversity analyses are needed. For effective use, however, the existing genetic resources need to be characterized into appropriate groups for utilization in future breeding programs. In fact, the breeding programs require a dynamic working collection and new genetic materials, possessing desirable genetic variability from time to time. The analysis of diversity in the available germplasm is also important for effective and efficient management and utilization of the plant genetic resources (Frankel *et al.*, 1995). Such analysis is essential not only for the identification of different collections but also to determine their genetic relatedness. The information generated could be successfully used in plant breeding programs. It is also relevant in the present context of intellectual property rights and trade agreements.

According to Gill (1989), germplasm utilization of several crops including linseed is limited in many developing countries, like Ethiopia due mainly to lack of proper evaluation and characterization, limited variants of useful traits, facilities, funds and trained manpower. Proper evaluation of the germplasm for stress conditions is limited because of inadequate facilities for artificial epiphytotics and for simulation of drought, heat, frost and other stresses. Similarly, screening germplasm for quality traits is frequently hampered because of lack of facilities. For example, there has been a strong need to develop linseed varieties with both low and high linolenic fatty acid types for different purposes. However, due to lack of laboratory facilities, it was not possible to analyze the available germplasm for fatty acid profiles.

Similarly, desirable information on the genetic make-up of useful variability in the existing germplasm is lacking (Frankel *et al.*, 1995). Only limited information is available on the number of genes controlling special traits, allelic relationships, linkages, etc. In addition, breeders have not properly classified the available germplasm for components of yield, disease and quality, which is essential for its utilization. Even if the collections are extensive, they may not be put to effective use due to the inadequate availability of useful genetic variants required by the plant breeders. Furthermore, many desirable traits occur in agronomically poor materials and breeders are usually reluctant to use such genotypes in their breeding programs due to the difficulty in recovering the desirable genotypes.

Lack of facilities for data storage and retrieval has also been one of the major problems for the classification and evaluation of the crop germplasm (Gill, 1989). It was difficult to isolate the desired accession from a large collection due to limitations in data storage and retrieval facilities. The maintenance of large collections is difficult, as they require adequate funds, skilled manpower and long-term storage facilities. Although wild germplasm is known to possess useful variability for various characteristics, their utilization is limited because of different problems, like incompatibility barriers, undesirable linkages, lack of chromosome pairing and recombination (Allard, 1988).

Jones (1983) emphasized that in order to meet the germplasm needs of oilseed crops, the following functional activities are required sequentially, as they do for the major crop species as well: (i) Eco-geographic studies on distribution of genetic diversity. (ii) Acquisition of germplasm through exploration and exchange with other countries. (iii) Maintenance of the acquired materials with the major objective of avoiding loss of genetic diversity. (iv) Germplasm enhancement by showing their usefulness via transferring useful genes from exotic or wild types into agronomically acceptable backgrounds. (v) Evaluation through manipulating via all kinds of breeding techniques; screening under various environmental conditions to assess its value in terms of quality, physical and biological stress tolerances, and yield characteristics. (vi) Researching the maintenance of genetic diversity, which pervades the entire spectrum of activities from collection of samples to the use of that germplasm on farmers' fields.

The current globalisation and economic integration activities are a consequence of nations' and regions' growing interdependence. This may increase the threat to the diversity of genetic resources just as it does to the diversity of cultures and economic systems. This interdependence is not only geographical and economic, but also between generations and between biotechnology and biodiversity (Esquinas-Alcazar, 2002). Agricultural biodiversity is a vital inheritance from previous generations. The present generation also has a moral obligation to pass it on intact to his children so that they can meet unpredictable environmental changes and changing human needs. Because of population growth, the current and coming generations will have to intensify agricultural production. They will have more biotechnologies to choose from, but without biodiversity their options will be limited. Biodiversity provides the raw materials of genetic resources, as biotechnology provides the new tools to combine these raw materials into commercial varieties.

Is genetic diversity under serious threat? Yes, according to numerous reports (Ehrlich and Wilson, 1991; Eigenbrode, 1996; Esquinas-Alcazar, 2002). According to these authors, the world's biota is under siege, as a human population of about six billion places unprecedented pressures on the biosphere. Approximately 95% of the terrestrial surface is now occupied by human settlements or ecosystems managed for food and materials production (Eigenbrode, 1996). As a result, natural ecosystems are destroyed and fragmented, species are destroyed or doomed, and global genetic diversity is diminishing. The scale of this destruction directly threatens human civilization, which is dependent in numerous ways on biological diversity (Ehrlich and Wilson, 1991). The enormity of the crisis has stimulated international efforts to conserve biological diversity and to ensure sustainable use of its components. Moreover, half of the world's food today comes from just four plant species and five animal species and within those species there has been a tremendous loss of genetic diversity (Esquinas-Alcazar, 2002). Thus, we have to make sure that future generations have enough genetic diversity to sustain intensified agricultural production. Farmers of the developing countries live in much more fragile environments and economies, and one can see how a lack of genetic choice limits subsistence strategies. On the other hand, the poorest countries are the richest (Esquinas-Alcazar, 2002) in terms of the genetic diversity needed to

ensure human survival. The loss of biodiversity today undermines the food security of tomorrow and once we lost the genetic materials, we cannot get them back. Therefore, we have to invest our time, energy and other precious resources to further preserve, evaluate and develop them for the coming generations.

2.4 Values of genetic diversity for plant breeding programs

Plant breeding is essentially a selection of plants among the variables (Allard, 1988). An insight into the magnitude of variability present in a crop species is of utmost importance as it allows effective selection. The total observable variation, phenotypic variation, is made up of genetic and environment component of variations. Genotypic variation, the component of variation, which arises due to the genotypic difference and the base for selection is the main concern of plant breeders (Allard, 1988). Hence, in selection for yield more emphasis has to be placed on those attributes with low environmental variability. In other terms, genetic diversity is the foundation of all plant improvement programs. It is a measure of individual variation within a population and reflects the frequency of different types in the population (Frankel *et al.*, 1995). Diversity is derived from the wild progenitors, modified in response to cultivation and hence, it is a function of ancestry, geographic separation and adaptation to differing environments (Moll *et al.*, 1965).

Genetic diversity within a given plant population is a product of an interplay of biotic factors, physical environment, artificial selection and plant characters such as size, mating system, mutation, migration and dispersal (Frankel *et al.*, 1995). Harlan (1975) attributed the accumulation of genetic variation in the centres of diversity to artificial selection, environmental factors and the dynamics of hybridisation with the subsequent segregation and selection. Genetic diversity in domesticated crop species provides a source of variation which is raw material for the improvement of agricultural crops, and is essential to decrease crop vulnerability to abiotic and biotic stresses and to ensure long-term selection gain in genetic improvement and to promote rationale use of genetic resources (Smith and Smith, 1989; Martin *et al.*, 1991; Messmer *et al.*, 1993; Barrett and Kidwell, 1998). Recent literature survey (Dudnik *et al.*, 2001) on the extent of using plant genetic resources in research showed extensive uses of the conserved germplasm for research. About 42% of this study was undertaken on the assessment of genetic diversity among accessions,

while the studies on biotic resistances, breeding and molecular markers were 29, 26 and 20%, respectively.

Progress in plant breeding could be enhanced through a more complete knowledge of germplasm contribution and a thorough understanding of genetic relationships between genotypes in a given gene pool. Information about genetic diversity in the available germplasm is important for the optimal design of breeding programs. Therefore, the notion of genetic relationships among lines, populations or species has become an important tool for the effective management of genetic diversity in a given gene pool. Genetic distance estimates have been shown to be useful in many self-pollinated crops including linseed to: (a) examine the level of genetic diversity (effective population size) of a given germplasm pool (Murphy *et al.*, 1986; Souza and Sorrells, 1991; van Beuningen and Busch, 1997); (b) to monitor trends in germplasm usage (Cox *et al.*, 1986; Graner *et al.*, 1994); (c) to identify major groupings of related cultivars, breeding materials, and genetic resources (Messmer *et al.*, 1993; Graner *et al.*, 1994); (d) to select parents for establishing new base population (Bohn *et al.*, 1999); (e) for rational utilization of genetic resources (Graner *et al.*, 1994).

Many clear evidences (Allard, 1988; Harlan, 1992; Bohn *et al.*, 1999) show that the few plant introductions and races that developed crop husbandry, can hardly be expected to contain all genes of agronomic worth of any cultivated species. Additional genes of agronomic worth are available in foreign or exotic sources, especially as source of resistance for diseases and pests. Moreover, the future availability of exotic germplasm has been cited as a critical factor that will determine continued progress in raising genetic yield potential in various cops (Smith and Duvick, 1989; Rush, 1991). In other words, genetic diversity is practically worthless unless it encompasses genes that are useful, either in them, or in combination with other previously evaluated germplasm, in order to meet products required by farmers, processors and consumers.

All hybridisations start with considerations of several related elements such as germplasm, genotypic relationships and diversity. From this process emerges decisions that are translated into beginning plant-breeding operations, namely, choice of parents and that of possible crossing patterns. Most breeders are linked

with germplasm projects that represent cooperative participation in preparation and release of germplasm. For instance, Jensen (1962) proposed a world germplasm bank for cereals to be founded on breeder contributions of surplus F_2 embryo seeds of each crop, which could be mixed annually with the previous stockpile. Suitable maintenance procedures would keep the composite available for distribution to any interested breeder. The International Plant Genetic Resource Institute (IPGRI) can handle such operations in order to make it more useful and effective.

Genetic conservation and the future of plant breeding have been emphasizing the general view of the narrowing genetic base in some well known crops, like wheat, rice, potato, etc. Information on composite crosses of wheat, barley, etc. is generally available (Jensen, 1988). A good piece of detective analysis of Qaulset (1975) illuminates both history of plant exploration and the value of germplasm collections. His analysis was focused on barley collections from Ethiopia. The Ethiopian barley, once considered a centre of origin, is unique. The great ranges of geographic and climatic environments have allowed a comparable range of plant types. What particularly made Ethiopian barley unique was the discovery that their group alone in the world harbours resistance to an important worldwide barley disease, barley yellow dwarf virus (BYDV). Roughly one-fifth of the collected Ethiopian barley was resistant (Qaulset, 1975). The source or cause of the resistance genes for BYDV in Ethiopian barley was mutation (Jensen, 1988). Then, who knows if the germplasm of other crops, including that of linseed would contribute similarly, provided equal chances of proper collection and utilization efforts were undertaken? That is why collection should be a continuous process by covering areas far away from main roads, using appropriate collection guidelines and experienced workers.

Foreign genes from varieties that are exotic to a particular region including wild, weedy and alien species can provide increased genetic diversity to the currently used germplasm base. Exotic germplasm had been instrumental in the improvement of hybrid maize, wheat, sorghum, soybean, potato and tomato in the USA (Smith and Duvick, 1989). Breeders are never satisfied with only their past and current achievements, as they work in a biological environment where a new pest or disease can quickly reveal the risks resulting from complacency. That is why plant breeders

need to increase the amount of genetic diversity in breeding programs. Moreover, the few races of crops that are the foundation of developed agriculture hardly possess all genes of agronomic worth for future uses. Crop breeding programs, which are genetically broad based, should be able to provide ideal results. Trends of continuous gains under selection are expected from such programs (Smith and Duvick, 1989), while the narrowly based one would provide slow response to selection and increase the likelihood of crises triggered by outbreaks of diseases and insects. A shortage of genetic variability could exacerbate these deficiencies all over the world, threatening the usefulness of available varieties and the usefulness of breeding stocks.

Jensen (1988) has extracted some important findings from articles of Smith *et al.* (1978) on population structure and grain yield of crops, as the result of continued heavy selection pressure. The parameters of the gene pool structure encompassed open pollinations for several generations under high plant densities. There were three gene pools of different origin, namely, relating to degree of diversity, adaptation and number of previous cycles of recombination. Each year the gene pools were subjected to heavy selection pressure and the important findings were:

- Genetic variability decreased with generations due to selection pressures, even if not directional and intentional.
- Adaptive changes, for example, height and maturity, shifted to apparent coincidence with environments encountered.
- These major changes occurred in the first or second generation.
- Genetic variability was highest in the composite that incorporated adaptive materials.

Similarly, Smith *et al.* (1978) considered the effect of open-pollination for several generations, including the up-grading selection for grain yield and stability. The important findings from their second paper were:

- Significant interaction was found for the check hybrid x location but not for the gene pools x location. This may be interpreted as greater stability for gene pools (diverse genotypes and heterogeneous) as against the single crosses (one genotype per hybrid and homogeneous).

- More diverse germplasm in a gene pool increased its tolerance to high density, thus showing adaptability.
- Greater changes were seen in gene pools that had experienced fewer opportunities for recombination before this study was started in Canada.
- Grain yields varied in the gene pools; however, the proportions of genotypes with greater potential at better environments increased in all gene pools.

Hence, it might be expected somewhat the same things to happen in self-pollinated crops treated this way. The important point is to use this knowledge with emphasis on directed selection for traits and objectives of interest. Someone can encounter the expected reduction in genetic variance that will accompany heavy selection pressure by creating selection subsets. For instance, Fowlers and Gusta (1979) found that limited genetic variability existed in winter wheat breeding for winter hardiness. They observed little progress and improved varieties were only marginally superior, as the result of choosing among consistent survivors for the best performers. All in all, breeders, particularly with reference to broadening the germplasm base for desirable trait combinations, may describe the situation as one calling for greater attention.

Cox *et al.* (1985b) compared coefficients of parentage (the probability that a random allele at a random locus in the other individual) to similarity indices in four groups of soybeans using enzyme profiles and found that the two cultivars were identical at all loci compared. Similarly, Langer *et al.* (1978) published an article with important implication for broadening of germplasm base and choice of parents for crossing. They compared the relative yield performance of 66 wheat cultivars introduced since 1942. The results showed that there was a grand mean productivity advancement of 9% from 1932 to 1973, as measured against the mean of two checks, and this level was attained by the varieties developed from 1932 to 1942. But no further significant increase in productivity was found in the remaining three decades of the development periods. The authors postulated a small pool of genetic variability for yield in these varieties. Likewise, prediction and choice of parents, crosses, lines and cross quality level are related subjects for breeders in view of genetic diversity. Causes of cross quality differences are attributed to the diversity

of parents, that is, wide crosses, use of exotics and ways of crosses (single, double, three-way, back-crosses, recurrent selection and so forth). The highest yields and the greatest hybrid vigor in double crosses of different parentage emphasize the importance of diversity. Thorne and Fehr (1970) showed that three way crosses are an effective way to introduce exotic germplasm, such as plant introductions, into soybean breeding programs, and were superior to two-way crosses of adapted x exotic. In short, the genetic divergence of plants plays a vital role in the shaping up of successful breeding programs. Genetically diverse parents are likely to produce the high yielding and desirable segregates. Hence, quantitative assessments of genetic divergence are important aspects of determining the nature and extent of genetic variability in crop plants.

2.5 Size and structure of genetic resources

According to Chang (1989), whether a crop collection is called large or otherwise, should be based on the size of holding in relation to the total genetic diversity present in that crop and its relatives. The size of a germplasm collection is related to the mandate of the institution and operational and managerial aspects. The comprehensiveness of a crop collection should be determined by a group of researchers specializing in that crop after a thorough assessment of available information and supplemented by field survey. Adequate inventory is necessary to ensure for distinct representation of the accessions, without overlooking and duplications. For example, the general criteria used in rice inventory included variety name or its code, country of origin or seed source, other passport data and key morpho-agronomic traits. The distinct accessions need to be kept separately, while the obvious duplicates are bulked, using appropriate statistical tests, like the clustering method. Electrophoresis is also useful in classification processes. New collection expectations and donations have to be encouraged and proper storage facilities, such as cold stores are also needed.

Several workers (Chang *et al.*, 1982; McNaught, 1988; Chang, 1989; Smith and Duvick, 1989) have indicated the genetic erosions in gene banks as a result of the following factors. (1) Shift in policy program or funding (2) turnover in personnel (3) lack or broken storage facilities (4) unexpected setbacks in regeneration or processing operations (5) unexpected natural disasters. Thus, number of accessions

is only a guide and is meaningless in estimating genetic diversity. According to Chang (1989), the advantages of large collections are as follows. A large collection is generally more diverse in genetic composition and more comprehensive in eco-geographic coverage, given the redundant accessions have been removed to the possible extent. If well planned from the beginning and careful field collection is implemented, a large collection can be expected to have a rich accumulation of useful alleles and a high frequency of such alleles in the collection (Marshall and Brown, 1975). Moreover, a large collection can supply accessions with several desired traits in different genetic backgrounds. For an important stable food crop of the humid tropics such as rice, the area of cultivation is still expanding into new ecosystems as in Africa and South America and the intensity of multiple cropping is also increasing rapidly (Chang, 1989).

The changing situation in pest damage, and edaphic and other ecological stresses require a broad spectrum of genetic diversity to provide genetic protection. A large collection requires adequate physical facilities, a broader range of scientific staff, greater financial support, and stronger supporting services than a small collection demand (Chang, 1989). Regarding the efficacy of facilities and operations related to large collections, it is more efficient to build, equip and operate facilities that will accommodate a large collection than small one. Generally, greater initial investments in building the facilities and storage equipment may lead to lower operating costs over a period of long time (Chang, 1989). In terms of operations, a large collection requires more germplasm-oriented workers and efficient supporting staff. Implementing on-job training of more competent and experienced staff members can upgrade working force. A larger collection can also provide its workers with greater opportunities to carry out diversified research and thus increase the incentives for advancement.

In general, Chang (1989) has summarized the following points from his experience and study with a large and diverse rice germplasm collection.

1. A large collection is very effective in furnishing useful genes and rare alleles. However, the size should refer to comprehensiveness in genetic diversity rather than numerical size.

2. Production of crops, like rice under irrigated culture and continuous multiple cropping in the tropics has led to rapid changes in varietal turnover, pest dominance and composition of pest population. The expanding cultivation and the dynamic changes in crop ecosystems call for new genes and a greater diversity.
3. Continuous growth in a large collection should be allowed to conserve additions having distinctiveness. Meanwhile, serious efforts should be made to trim redundancy so that the conservation of inputs would be more efficient.
4. Medium-sized national collections may double as regional collections to backup the base collection.
5. Wild relatives of crops should be further collected to add to the usefulness of the collections.
6. Conservation must be accompanied by multidisciplinary and systematic evaluation, full documentation, and effective communication among different disciplines, so as to enhance the usefulness of large collections and to justify long-term investments.
7. Much of the potential usefulness of genetic resources lies in the yet untested materials.

2.6 Major principles in diversity analysis

The principal ideas behind the concept of base and core collections are important. Base collection is multiplied at least once and conserved in its entirety. However, characterization and evaluation of large collections is costly and time-consuming (Frankel, 1989). Consequently, there is an obvious need for reduction to establish a core collection based on the following two objectives: to have a manageable collection to the need of breeders and other users; and to include the widest possible range of variability.

According to Frankel (1989), the cultivation system used is an important source of information and becomes more important when the crop has a long tradition in a particular country. The quality of the information obtained from the collection site is an extremely important factor (Harlan, 1975; Hamon and van Sloten, 1989). The

information on the geographic co-ordinates is absolutely necessary and it is possible to improve the quality of information during collection by concentrating on the following major issues:

- Restricting the collection mission to one or a very limited number of crop species;
- Taking sufficient time to become familiar with the local conditions and customs;
- Acquiring a systematic translation of local names;
- Ensuring the involvement of local farmers, especially women farmers.

Reliable and relevant evaluation of potential parents forms the corner stone of all breeding programs. Accordingly, evaluation of each crop needs to be divided into two categories (Fischbeck, 1989). These are the ones, which are largely or wholly carried out by the gene bank staff (characterization) and the ones done by the plant breeders, pathologists, entomologists etc., (i.e., in depth evaluation). The latter implies identification of genetic bases of the materials. The improvement in yield will have to be measured more in terms of combining ability than in terms of actual yield or excessive formation of individual yield components (Frankel and Brown, 1984). All in all, evaluation needs much more support of resources in money and other forms, concentrating on more promising accessions and also on pre-breeding activities. Evaluating larger numbers of accessions has to be a major undertaking with concerted efforts of breeders to identify many valuable accessions via developing proper screening techniques and locations to handle large numbers of accession and to give reliable results. Priorities should be given to evaluate the primary and secondary gene pools as an essential prerequisite to their use in the breeding programs. Germplasm collections are screened for resistance or tolerance to the major yield limiting factors. These are diseases, drought, insect pests and nutritional factors. Accessions are also screened for yield potential, seed size, oil content and time to maturity. Intensive screening techniques are required both in the field and laboratory as well. In this regard, Williams (1989) outlined the following practical considerations used to facilitate the evaluation of accessions and to accelerate their utilization.

- Careful acquisition of passport data, assessment of gaps and their fulfilling through purposeful collection.
- Characterization and evaluation of accessions as major prerequisites for use in breeding.
- Bilateral agreements with others holding the same or similar to partition responsibility for characterization and evaluation and for safe duplication.
- Clear links with breeders and other collaborators such as with laboratories specialising in related scientific research.

The ability of curators to respond helpfully to the requests of breeders for material clearly depends on the adequate description of accessions and the ability to manipulate the information in the computer database. Most collection should be carried out in co-operation with breeders or other scientists. Because of environmental sensitivity, evaluation of data may have little relevance unless detailed records of growing conditions can aid the interpretation and application of the results. Well-organized databases are clearly essential and the data they contain should meet the needs of curators, breeders and other research scientists.

The other approach of improving the usefulness of gene banks to breeders could be through the establishment of core collections, which could include a representation of genetic diversity in the collection as a whole. For the promotion of characterization and evaluation, different skills and scientific expertise are required. Consequently, competent staff is more important than expensive buildings and equipment (Williams, 1989). The same author has also pointed out that efficient evaluation and more extensive use of germplasm collection will proceed when:

- Gene bank collections are well managed and documented;
- There are comprehensive crop databases which combine national collections into regional or international groups;
- Strategies are determined by the collaboration of experts knowledgeable on the crop;
- There are clear and good working relationships between the gene banks, breeders and other scientific users. In a nutshell, if these practical considerations

have received adequate attention, significant advance in meeting agricultural needs will be expected via utilizing the germplasm collections in the breeding programs.

Frankel (1989) indicated that evaluation of germplasm is an essential preliminary activity for utilization. It was seen as an organized and institutionalised activity resulting in information, which comes to the users, like breeders as standardized and computerized documentation. Evaluation was the responsibility of the curators of germplasm collections. The characteristics to be evaluated were nominated by specialists in various crops. This was the basic strategy of germplasm collection and evaluation Frankel (1989). Breeders were looking to the gene banks for information on agronomically useful traits (Duvick, 1984; Frankel, 1989). The latter author indicated that plant breeders are not using gene banks very widely due mainly to the scarcity of information that was of use to the individual breeder. Subsequently, a significant proportion of breeders turn to germplasm collections when the available ones do not provide the genes they require. That means, breeders need to be involved in the collection and evaluation processes in order to exploit fully the potential of the existing genetic resources.

According to Frankel (1989), more effective evaluation was realised to come from institutions where there is a close organizational and personal contact between curators and breeders, and where breeding objectives are reflected in the evaluation program. Hence, breeders need to take active part in the process, especially when the breeding objectives are diverse and competitive, as only the concerned breeders can determine the genes or characters they want to introduce into their breeding materials. Since aims of breeding change rapidly, evaluation needs to be adapted accordingly. Moreover, breeders can recognize which characters need to be evaluated or looked into under their own environments. Frankel (1989) also enumerated the responsibilities of curators in collecting and disseminating information as follows:

- Obtaining information on the origin of accessions (passport data) from collector, breeder or other sources.
- Characterization.

- Rationalizing of the collection to reduce the task of evaluation to a manageable scale, like a core collection.
- Organizing and co-ordinating the participation of relevant specialists, such as plant pathologists, entomologists, etc.
- Co-ordinating and making available the information that results from the evaluation process without being directly involved in the pre-breeding activity.

The four status and modes of accessions' utilization (wild, domestication, plant and gene introduction) need to contain information of origin (passport data), characterization and evaluation data. Information on collection sites (origin on map, latitude and altitude) of accessions is important for all purposes of genetic resources (Chang *et al.*, 1982). Site identification is the most significant evidence available to the curator for designating a core collection and great help in identifying duplicates. It is essential information for eco-biological, evolutionary or population genetic research and for planning further collection.

Records on topography or soil characteristics are also valuable for plant breeders concerned to improve adaptation to particular conditions or tolerance of edaphic or climatic stresses (Clawson, 1985). There is increasing emphasis in the world on seeking genes for tolerance to specific adverse environmental factors such as waterlogging, soil acidity, specific mineral deficiencies or toxicities, besides climatic stresses. In general, descriptive of the environment including the associated flora, hydrology, the land use system and eco-geographic information are important for assessing redundancy and representatives of research fields (Frankel, 1989).

Characterization consists of recording those characters, which are highly heritable, can be easily seen by the eye and expressed in all environments (Frankel and Brown, 1984). It is basically an account of the morphology of plants throughout their development or at maturity. Characterization data are useful in management measures, such as the designation of a core. It should provide a standardized record of readily assessable plant characters that together with passport data go a long way to identify an accession. Characters that cannot be seen, such as isoenzyme data can usefully complement characterization for the purpose of classification.

Characterization by curators can be of considerable help to the breeder through providing a preliminary account of physiological baseline data such as, tillering, times of flowering and maturity, which would help to narrow the selection of potential breeding stocks. It is generally, regarded as a responsibility of curators (Chang, 1989).

On the other hand, evaluation of genetic resources is a muli-dimensional endeavour, involving scientific fields as diverse as crop cyto-genetics and evolution, physiology, pathology and agronomy. These collaborating disciplines contribute information that bears on the choice and utilization of genetic resources by the breeders. Generally, multi-disciplinary participation of genetics, plant physiology, pathology, entomology, bio-geography and biochemistry is essential (Frankel, 1989).

According to Frankel (1947), the characters of concern to plant breeders were broadly divided into two types: observable (strongly inherited and expressed) and variable (complex) characters. The former can be readily identified and/or selected in single plants or their immediate progenies. They are expressed under normal growing conditions of the crop or they may require special conditions or expression such as a specific parasite or environmental stress. The latter is subject to environmental variation. They are largely responsible for differences in yield or adaptation. Inheritance is polygenic and evaluation may require replicated tests in multiple sites. Observable characters can be categorized into three major groups.

1. Morphological, physiological or biochemical traits relating to survival, productivity or quality.
2. Resistance to diseases and pests
3. Tolerance of adverse conditions or stresses

Curators, breeders and other relevant professional under appropriate environments should study the first observable characters. Similarly, plant pathologists and entomologists under the genetics and ecology of host-pathogen relations have to evaluate the resistance of pests. The tolerance of adverse conditions or stresses such as high and low temperature, drought, frost, waterlogging, soil acidity, salinity, nutritional deficiencies or toxicities are mostly polygenic, against the oligogenic

pathogen resistance (Frankel, 1989). Appropriate field conditions and laboratory tests are crucial for evaluating stress tolerant accessions. Pre-breeding which is the early phase of any breeding program makes use of the exotic germplasm. Breeders who lack human and capital resources, like those in developing countries, can rely on pre-breeding by exploiting exotic germplasm via transferring valuable genes from them into well-adapted ones. In short, the roles of characterization and evaluation in the utilization of genetic resources is currently well recognised, the responsibilities of curators being to maintain, characterize and transmit the data to data banks. Likewise, the participation of breeders is essential, from defining the objectives to the checking of evaluation results. Furthermore, Chapman (1983) also concluded the following strategies in the collection of wild relatives of crops, to capture maximum genetic variation and thereby to reconcile and satisfy needs of plant breeding:

- Giving priority for those most easily utilized and endangered;
- Choosing the target area of collection;
- Collecting from diversified geographical and ecological sites; and,
- Giving more emphasis for number of collection sites than for accessions.

2.7 Approaches of diversity analyses

Knowledge about genetic relationships among cultivars is usually obtained indirectly from eco-geographic information about the genotypes, pedigree and heterosis data, and directly from plant characteristic data such as morphological traits, biochemical data, and more recently, from DNA based marker data (Kumar, 1999). Before the new tools of DNA markers came on the scene, the conservation and utilization of genetic resources have been using massive approaches at all stages. The traditional approach has been using mass collection, mass screening, mass crossing and selection, whereas the new approach is applying core collection, targeted gene tagging and transferring techniques (Dar, 2001). The former was costly, time consuming and thus had limited progress, while in the latter approach, one is learning how to be much more targeted at all stages of collection, gene discovery, and moving the genes into good agronomic backgrounds. Preliminary and more detailed data evaluation could be done, following the choice of potentially

useful accessions. In the former, information on basic agronomic characters such as maturation time, plant height, germination percentage and disease resistance are collected, whereas simply inherited traits (seed quality, reactions to insects and diseases) and complexly inherited traits (tolerance to heat and drought stress, combining ability and yield) are covered under more detailed evaluations.

2.7.1 Morphological

Traditionally, genetic diversity estimates in crop species are based on differences in morphological characters and quantitative traits (Schut *et al.*, 1997). Typically, genotypes are grown in the field or greenhouse, and estimates of relationships are based on the range of expression of various traits among genotypes. When phenotypic estimates are used to represent the degree of genetic relationship between two lines or populations, it is assumed that similarity in phenotype accurately reflects similarity in genotype (Cox *et al.*, 1985a). This approach has been extensively used in genetic similarity and diversity studies (van Beuningen and Bush, 1997). Morphological traits continue to be the first useful step in the studies of genetic relationships in most breeding programs because: (a) the existing data bases on the germplasm collection or breeding stocks can often be used for genetic analysis; (b) statistical procedures for morphological trait analysis are readily available; (c) morphological information is essential in understanding the ideotype-performance relationships; and (d) explanations of heterosis may be enhanced if morphological measures of distance included as an independent variable (Cox and Murphy, 1990; van Beuningen and Busch, 1997).

However, use of morphological traits for the study of genetic relationship has been criticised. Genetic relationship evaluation among germplasm using morphological characteristics are lengthy and costly processes (Cooke, 1984). The genetic control of many morphological characters is assumed to be complex, often involving epistatic interactions, and has often not been elucidated (Smith and Smith, 1989). Many morphological markers are recessive and therefore only expressed in the homologous condition. Most elite cultivated and breeding materials do not abound with an array of readily observable morphological markers, a large number of which have deleterious effects on agronomic performance (Smith, 1986). Furthermore, most morphological attributes are subject to large genotype x

environment interaction effects (Kumar, 1999). Hence, morphological appearance cannot adequately describe cultivars without extensive replicated trials (Lin and Binns, 1994) and, therefore, valid comparisons are only possible for descriptions taken at the same location during the same season (Smith and Smith, 1989).

2.7.2 Biochemical/ molecular

2.7.2.1 Isozymes

Direct measures of genetic similarity between individuals have been determined from isozyme markers in many crop plants (Brown, 1979). Isozymes are variants of the same enzyme having identical or similar functions, but differing in electrophoretic mobility. They reveal differences in the gene sequence and function as co-dominant marker (Kumar, 1999). Isozyme data can be used to quantify similarities and differences between genotypes because: (a) isozyme surveys represent a basic level of investigation for species that are poorly documented; (b) isozymes are universal in a sense that estimates of the extent of distribution of genetic diversity can be directly compared between individuals, populations, or species; and (c) isozyme methods are appropriate to investigate genetic variation from large samples of individuals because the procedure is fairly quick, simple and inexpensive, and interpretation is relatively easy (Tanksley, 1983; Cooke, 1984).

Nevertheless, enzyme-encoding loci do not constitute a random sample of genes, and they are not randomly dispersed through the genome. Some isozyme variants are not selectively natural and electrophoresis will detect only a portion of the actual variability present in amino acid sequence (Bretting and Widrechner, 1995). Hence, isozyme data, although they provide new insights into genetic relatedness among elite breeding materials, their usefulness for obtaining reliable estimates is generally limited by the insufficient sampling of the genome (Melchinger *et al.*, 1991), small number of loci and low degree of polymorphism among closely related genotypes (Messmer *et al.*, 1992). Furthermore, isozyme expression can be significantly influenced by the environmental factors and management practices and by plant development stage (Bellamy *et al.*, 1996). Generally speaking, although isozyme analysis is relatively inexpensive and easy to handle, it is not as useful as

DNA markers due to the low level of polymorphism and limited number of loci (Bernatzky and Tanksley, 1989).

2.7.2.2 Storage proteins

Storage proteins are the primary products of structural genes, which, in turn, are coupled into genetic systems, can serve as markers for the genes that encoded them and the system they are located in, which may be a set of genes, chromosome or genome as a whole (Cooke, 1984; Konarev *et al.*, 1979). Polymorphism of storage proteins, as manifested in a variety of molecular forms, is evident from various types of electrophoretic techniques that detect charge and size differences (Konarev *et al.*, 1979). The various types of electrophoretic methods available for protein analysis includes one dimensional starch gel electrophoresis, polyacrylamide gel electrophoresis (PAGE: with or without sodium dodecyl sulfate: SDS), isoelectrophoresis (IEF), and two-dimentional polyacrylamide gel electrophoresis (Cooke, 1984; Wrigley, 1992). Polyacrylamide gel electrophoresis in the presence of SDS has been world wide used for the analysis of wheat seed storage proteins (Wrigley, 1992; Galili and Feldman, 1983). This technique is rapid, relatively low cost, and capable to handle a large number of samples (Gept, 1990).

Seed storage protein markers have been indicated to be tightly linked with many important agronomic characters such as seed size, glume colour and pubescence, heading time, disease and pest resistance, frost hardiness and plant height (Konarev *et al.*, 1979; Metakovsky *et al.*, 1990). Consequently, seed storage proteins have been used as markers for the analysis of genetic diversity within and among populations (Gregova *et al.*, 1997). In wheat breeding, seed storage protein markers have been effectively used for accurate cultivar identification (Cooke, 1984; Wrigley, 1992), selection of quality types (Payne, 1987), pedigree verification (Wrigley and Shepherd, 1977), production of pure foundation seed, prediction of heterotic combinations and in the studies of the pattern and the level of genetic diversity and genetic relationship among adapted cultivars (Cox *et al.*, 1985a; Labuschagne *et al.*, 2000). The variation and amount and type of seed storage proteins is the main factor responsible for determining the differences in bread- and pasta-making quality and nutritional properties of flour derived from different wheat varieties (Payne, 1987; Lafiandra *et al.*, 1993). Protein markers could also be

useful in the protection of intellectual property of new cultivars and documentation and variety description required in Plant Breeders Rights' application (Cooke, 1984; Wrigley, 1992.). The effectiveness of storage protein markers as a means of classifying adapted cultivars and populations has limited the extent of isozyme marker application in wheat and barley breeding programs (Cooke, 1984).

2.7.2.3 DNA markers

Among all the various approaches used till now for estimation of genetic diversity and relatedness in plants, the DNA methods appear very promising, as demonstrated in a number of recent publications (Paterson *et al.*, 1991; Weising *et al.*, 1995; Karp *et al.*, 1996; Kumar 1999). A variety of DNA-based markers have been developed for measuring genetic diversity in crop species (Schut *et al.*, 1997). DNA markers have proven to be powerful tools in the assessment of genetic variation both within and between plant populations and in the elucidation of genetic relationships among adapted cultivars and accessions (Karp *et al.*, 1996). Compared to morphological and biochemical markers, the DNA marker approaches are highly informative because: (a) they allow direct comparison of genetic diversity to be made at the DNA level; (b) they have the potential to identify a large number of polymorphic loci with an excellent coverage of an entire genome; (c) they are phenotypically neutral; (d) they allow scoring of plants at any development stage; and (e) they are not modified by environment and management practices (Tanksley *et al.*, 1989; Messmer *et al.*, 1993).

The DNA techniques have been used to investigate the extent of genetic diversity and genetic relationships within and between cultivars and elite materials of most crops. DNA markers directly measure DNA sequence variation among genotypes, though results may be confounded by biased or incomplete genome coverage, detection of comigrating nonhomologous fragments, or high crossover frequency between markers used in the evaluation and linked genetic material (Barrett and Kidwell, 1998). Some DNA markers, such as RFLP also require the use of hazardous radioactive isotopes (Tanksley *et al*, 1989; Bohn *et al.*, 1999). Despite the great power they offer, DNA marker techniques are relatively expensive, so that sample sizes need to be small (Melchinger *et al.*, 1991). However, the recently developed PCR-based markers, such as AFLPs and SSRs are playing increasingly

important roles in genetic diversity analysis of plants for they are generally simpler, more sensitive and automated than RFLPs and the traditional morphological methods (Kumar, 1999). Therefore, molecular markers are useful as they are naturally occurring, have very few negative effects on phenotype, are co-dominant and are not subject to environmental influence (Bernatzky and Tanksley, 1989).

2.7.2.4 Fats and fatty acid profiles

Seed storage lipids are the major source of edible vegetable oils, the quality of which is dependent primarily on their fatty acid composition (Green, 1986a). Of particular importance is the relative proportion of the unsaturated fatty acids namely oleic, linoleic and linolenic acids. These fatty acids determine the end use of the oil, those being high in linoleic are being utilised in polyunsaturated oils and margarines, while those that are high in oleic acid are more suited to cooking and salad oils. Oils having high levels of linolenic acid, such as that of linseed are unsuitable for use in edible products due to the problems of flavour reversion associated with the autoxidation of this unstable fatty acid. Hence, quality of oil depends upon its fatty acid composition.

Batta *et al.* (1985) assessed 86 genotypes of linseed for oil content and fatty acid composition. Their result indicated wide variability among the genotypes. Oil content varied from 37.8% to 47.7%. Oleic acid ranged from 15.7% to 33.5%, linoleic acid from 7.56% to 15.3% and linolenic acid from 40.61% to 62.58%. They also reported a positive correlation between oil content and linolenic acid and a strong negative correlation between oleic and linolenic acids. Eventually, they indicated the possibility of breeding linseed varieties containing low and high linolenic fatty acids for different purposes. A similar study (Khotpal *et al.*, 1997) revealed that the total lipid content of five linseed cultivars varied from 42.1% to 46.3% whereas neutral lipid, glycolipid and phospholipid contents were 87.8-89.6%, 5.8-6.6% and 3.8-5.8%, respectively. The neutral lipids were separated into monoacylglycerol, diacylglycerol, triacylglycerol, free fatty acids, sterols, steryl esters and hydrocarbons. The major fatty acids were reportedly linolenic, linoleic, oleic, stearic and palmitic acids (Khotpal *et al.*, 1997).

An investigation carried out in Australia (Green and Marshall, 1981) showed significant variation in oil content and fatty acid composition between and within varieties in a diverse collection of 214 linseed accessions. Lines that had up to 46% oil were identified as compared to 40% of the standard cultivar, and high oil content was consistently associated with larger seeds. Oleic acid and linolenic acid varied between 13.3 and 25.2% and 45.5 and 64.2%, respectively, both were strongly negatively correlated between all tested varieties. This inverse relationship implied the synthesis of linolenic acid in linseed in the following sequence: Oleic acid → linoleic acid → linolenic acid

Studies concerning the inheritance of oil content suggested that oil content is inherited as a quantitative character, with heritability estimates between 66.4 and 67.2% (Kenaschuk, 1975). The same author indicated that both oil quantity and quality were highly heritable in linseed. He further indicated the presence of additive gene action at one location and non-allelic at another location. That is to say, identical genotypes can exhibit different types of gene action when grown in different environments (Kenaschuk, 1975).

The ratio of fatty acid components can change with the environmental conditions or it reacts to different climatic conditions during ripening (Table 2.1) and this determines the physicochemical properties of oils. Although oil content and quality are highly heritable traits in linseed, environment has a marked influence (Kenaschuk, 1975). Cool climates delay maturity of and provide a longer period for oil and fatty acid synthesis. The adverse effect of high temperature, especially above 32%, on the oil content of linseed has been reported (Kenaschuk, 1975; Green, 1986a). A warm climate favours the formation of saturated fatty acids and acids with one double bond, while cold climate favours the formation of unsaturated acids with two or three double bonds. Linolenic acid was considered to be the most sensitive to climatic conditions. Photoperiod influences both oil content and quality of linseed (Kenaschuk, 1975). Oil content, iodine number and linolenic acid percentages increased progressively with longer photoperiods. The other environmental factors that affect the variability of oil content and oil quality are soil moisture, soil fertility, plant spacing and the presence of disease, according to Kenaschuk (1975).

Mutagenesis and subsequent selection have resulted in the production of linseed varieties, known as 'Linola' types, with a linolenic acid content of the seed oil of around 2% and a linoleic acid content of around 70% (Uppstrom, 1998). Oil from 'Linola' types can be used in food products for human consumption. Work on Linola types was undertaken with a view to developing new varieties high in linoleic acid with economic value attached to the other plant parts and products. Attempts to alter the fatty acid composition of the oil through interspecific hybridisations were unsuccessful, according to Uppstrom (1998).

Table 2.1. Composition of linseed oils, as affected by different climatic conditions (Robbelen, 1975)

Grown in	Iodine value	Component fatty acids			
		Saturated	18:1	18:2	18:3
Nebraska	155.4	12	32	21	35
Minnesota	162.8	11	30	18	40
N. Dakota	164.7	10	34	12	44
S. Dakota	171.5	10	27	19	44
Montana	177.0	10	25	18	47
Oregon	182.4	9	22	20	49
Saskatchewan	187.0	9	22	15	54
Nova Scotia	196.0	9	16	16	59

2.7.3 Other approaches and information sources

2.7.3.1 Eco-geographic information

Genetic diversity is not distributed uniformly throughout the range of geographic areas in which a taxon is grown. Information on eco-geographic origin of genotypes is therefore helpful in most genetic diversity studies (Frankel, 1989; Bechere *et al.*, 1996). Current evidence from allozyme studies suggests that geographic distribution accounts for most of the observed variation in wild crop species (Hamrik and Godt, 1990). In cultivated crop species, geographical distribution patterns reflect both the specific selection pressures prevailing in a particular environment as well as history (Hawtin *et al.*, 1997). Agro-geographical information is specifically used when other information on genotypes is either not available or else is very sparse and is often applied for gene-bank materials (Schut *et al.*, 1997).

2.7.3.2 Parentage analysis

When pedigree records are available, parentage analysis is the most widely used and least expensive indirect measure of genetic diversity and genetic relationship among cultivars in various autogamous crops (Cox *et al*, 1985b; Martin *et al.*, 1991; van Beuningen and Busch, 1997). Pedigrees of the varieties and lines are often traced back to landraces and wild accessions. Coefficients of parentage (COP) summarize geneological information from an array of cultivars. Originally devised by Wright in 1922 and Melecot in 1948 (Souza and Sorrells, 1989), the COP between two cultivars is defined as the probability that a random allele taken from a random locus in one cultivar is identical by descent to a random allele present at that same locus in the second cultivar (Kempthorne, 1969; Cox *et al.*, 1985b). In this context, COP can be used as an index of genetic relationship among cultivars, with values ranging from 0, where two cultivars are completely unrelated and hence no alleles in common, to 1, where two cultivars share all alleles in common. COP analysis has been applied in several crop species including soybean (Cox *et al.*, 1985c; Sneller, 1994), wheat (Cox *et al.*, 1985a; van Beuningen and Busch, 1997) and barley (Martin *et al.*, 1991). Cox *et al.* (1986) monitored the change of genetic diversity of these two gene pools using COP values weighted by the acreage data of cultivars in a given year. The genetic relatedness of the U.S. soft red winter wheat gene pool has not changed remarkably when measured by acreage-weighted COP, ranging from 0.30 in 1919 to 0.22 in 1984. On the other hand, mean acreage-

weighted COP within the hard red winter wheat gene pool has significantly declined from 1.0 to 0.4 in the same period. By similar analysis, oat gene pool in U.S. was found expanding over time (Souza and Sorrells, 1989).

Accuracy of COP analysis depends on the availability of reliable and detailed pedigree records for all cultivars in the study. However, for modern cultivars pedigrees are increasingly becoming complex because unadapted and wild germplasm sources from diverse geographic regions have been introgressed into elite germplasm for new resistance gene to fungal and viral, but their true genetic contributions are unknown. Also, private breeding companies have protected pedigrees of the modern cultivars as trade secret. Under these conditions, calculation of COP is often not feasible (Graner *et al.*, 1994; Melchinger *et al.*, 1994). Furthermore, estimates of relationships based on COP might be incorrect because of inadequate simplifications in understanding the model that assumes no relation amongst original ancestors of the relevant gene pool, equal parental contributions, no selection pressure, no mutation and no random genetic drift (Messmer *et al.*, 1993; Barrett and Kidwell, 1998)

2.7.3.3 Heterosis record

Heterosis data would also provide indirect estimates of genetic relatedness among cultivars and breeding lines (Smith and Smith, 1989). The co-ancestries within a heterotic group (i.e. collection of closely related inbred lines) are usually high, whereas the co-ancestries between two heterotic groups comprising a heterotic pattern are usually low. These data presumed to survey numerous loci that are widely spread throughout the genome; the precise locations and magnitudinal effects of those loci are, as yet, unknown. They have shown relationships between lines that closely mirror those to be expected on the basis of unknown pedigree (Smith and Smith, 1989). For these reasons, heterosis is considered to be an indicator of genetic relationships, at least across a limited range of germplasm as would usually be the case with elite breeding materials (Moll *et al.*, 1965).

2.8 Measurements of genetic distance

The pattern and the level of genetic diversity in a given crop gene pool can be measured in terms of genetic distances. Genetic distances are measures from the

average genetic divergence between cultivars or populations (Souza and Sorrells, 1989). Moll *et al.* (1965) defined genetic divergence of two varieties as a function of their ancestry, geographic separation, and adaptation at differing environments. Genetic distance is the extent of gene differences between cultivars, as measured by alleles frequencies at a sample of loci (Nei, 1987). Genetic similarity is the converse of genetic distances, i.e., the extent of gene similarities among cultivars. The measure of distance or similarity among cultivars is the covariance of allele frequencies summed for all characters (Smith, 1984).

Measures of genetic proximity or relationship can be derived from genetic marker data and coefficients of parentage (COP). Several genetic distance measures have been used to quantify genetic relationships among cultivars or germplasm accessions. Each variable of molecular marker bands such as isozymes, storage proteins and DNA-based marker bands are considered a locus so that every locus has two alleles. Banding profiles of each line or cultivar can be scored as present (1) or absent (0). Pair wise binary matrices are constructed from the arrays of 1s and 0s to calculate genetic distances based on a range of formulae. One of the most useful genetic distance formulae is that of Euclidean distance, which is the square root of the sum of squares of the distances between the multidimensional space values of the distances for any two cultivars (Kaufman and Rousseeuw, 1990) and it can be written as: $GD = \sqrt{\sum[(X_i - Y_i)^2 / N]}$, where GD is the genetic distance between individual X and individual Y; i = 1 to N; N is the total number of bands, and X_i and Y_i are i^{th} band scores (1 or 0) for individual Xs and Ys. The other measures of genetic distance include Manhattan distance (Kaufman and Rousseeuw, 1990) and Roger's distance or Modified Roger's distance (*MRD*) (Rogers, 1972). Genetic distance has been also generated from several genetic similarity indices (*GS*) that can be calculated as *1-GS*. One useful similarity index is that of Nei and Li (1979): $GD = 1 - [2N_{xy} / N_x + N_y]$, here $2N_{xy}$ is the number of shared bands, and the Nx and Ny are the number of bands observed in individual x and individual y, respectively. Other similarity indices such as Jaccard's (Rohlf, 1993) and Gower's similarity coefficients (Gower, 1971) have been extensively used in genetic distance determination (Barrett and Kidwell, 1998; Barrett *et al.*, 1998). Genetic distances have been also determined from coefficients of parentage (*COP*) obtained from

cultivar pedigree documents, as *1-COP* (Cox *et al.*, 1986; Graner *et al.*, 1994; van Beuningen and Bush, 1997; Barrett *et al.*, 1998). *COP* is the covariance of allele frequencies between cultivars as determined by identity of parentage (Cox *et al*, 1985b).

The pattern of genetic relationship or proximity among cultivars can be conveniently shown by multivariate techniques such as cluster analysis or ordination analysis. Clustering techniques can present complex, multidimensional patterns of diversity (Sneller, 1994). Clustering is a useful tool for studying the relationships among closely related cultivars or accessions. In cluster analysis, cultivars or lines are arranged in hierarchy by agglomerative algorithm according to the structure of a complex pair-wise genetic proximity measures. The hierarchies emerging from cluster analysis are highly dependent on the proximity measures and clustering algorithm used (Kaufman and Rousseeuw, 1990; Hintze, 1998). Five different clustering algorithms are available in NCSS 2000 computer package for cluster analyses (Hintze, 1998): (a) Single linkage, (b) the average linkage method called Unweighted Pair Group Method using Arithmetic Average (UPGMA), (c) the average linkage method called Unweighted pair Group Method using Centroids (UPGMC), (d) Complete Linkage, and (e) Ward's method. Clustering technique, however, may not provide an insight into the underlying causes of patterns in genetic diversity.

In ordination techniques, the multidimensional variability in a pair-wise, inter marker proximity is depicted in one to several dimensions through eigen structure analysis. It permits the presentation of the clusters as points in Euclidean space (Murphy *et al.*, 1986). Ordination is best suited to revealing interactions and associations among cultivars or germplasm accessions which are described by continuous quantitative data (Bretting and Widrlechner, 1995). Principal component, principal coordinate, and linear discriminate analyses are the ordination techniques most frequently used in genetic relationships and cultivar classification studies (Murphy *et al.*, 1986; Schut *et al.*, 1997). Sneller (1994) has, however, indicated that the distances and patterns inferred from two dimensions can have limited validity if they do not account for a large proportion of the total variance in the data set.

In general, statistical parameters, such as univariate, bivariate and multivariate measurements can be applied to analyse the data of germplasm accessions. Univariate usually deals with descriptive statistics (minimum, maximum, mean, standard deviation and coefficient of variation) for both quantitative and qualitative characters, whereas bivariate presents correlation and regression of the collected data. Similarly, multivariate statistical tools are used to measure multiple variables simultaneously. These tools include, factor analysis, principal component analysis, discriminant analysis and hierarchical clustering method. Discriminant analysis is applied to fulfil two objectives (Hamon and van Sloten, 1989). The first is to test a classification of individuals into pre-defined groups according to a selected criterion. The second is to establish a classification of variables in descending order of discrimination, in order to select a minimum number, which will suffice. Generally, the reduction in the number of descriptors can be obtained through multivariate analysis. A step-wise approach consists of running a principal component factor analysis that projects the variability in a limited dimension. Then a clustering analysis is done which will be later tested by a discriminant analysis.

2.9 Limitations in using germplasm resources

Although there has been a dramatic increase in the number of gene banks and in the amount of samples held in these gene banks (Marshall, 1989), there are still important gaps in the existing collections. These gaps are in the areas of wild relatives of the major crops and in some regionally useful crops for which the IBGRC has been implementing a pragmatic and flexible program to fill these gaps (Hawkes, 1977). Nevertheless, the dramatic increase in material held in gene banks has not yet been matched by a concomitant increase in its use by plant breeders and other experimental workers. Consequently, there is a growing perception that genetic resource collections are generally underused (Marshall, 1989). The rapid growth in germplasm collections has been based on the premise that they represent a valuable resource for breeders and biologists. Their continuation could quickly diminish, if this premise comes into question because of lack of use, financial and political support.

According to Dudnik *et al.* (2001), the obstacles in using the plant genetic resources have been identified as lack of information on the germplasm, difficulty in

accessing collections, underproduction of seeds and the difficulty in transferring specific genes into good genetic backgrounds. Similarly, Frankel (1989) indicated that IBPGR has already identified the major constraints limiting germplasm use and tried to develop practical strategies to overcome the constraints. The constraints were many and varied; however, they were divided into two broad categories: those that limit the availability of germplasm and those that limit its uses provided that it is readily available. The first category includes economic constraints that are serious and common limitations to the availability of germplasm from collections, especially from national gene banks in developing countries (Frankel and Brown, 1984). Curators often have difficulty in obtaining sufficient funds to undertake seed increase of materials collected under IBPGR sponsorship for distribution to other gene banks and to users, particularly when the number of samples is large. This problem could be reduced in seed crops by collecting larger samples from the fields so that sufficient amounts would be available for both storage and distribution without the need for multiplication. Marshall and Brown (1975) indicated that the optimum sampling strategy, which can maximize the amount of useful genetic variability collected, is to sample 50 to 100 individuals per population and to sample as many as possible populations occupying as wide a range of environments as time and resources permit. Another economic constraint is funds required for sample preparation, packaging, distribution and plant quarantine regulations.

Legal constraints that included specific legislation restricting the export of propagating materials were also reported (Marshall, 1989) in prohibiting the export of propagating material of specific crop, which they consider to be of crucial importance to their national economies. Such legislation is generally in conflict with the principle of free availability of germplasm and it has been affecting industrial and plantation crops, like oil palm (Frankel and Brown, 1984). However, such restrictive legislation is believed to diminish in the foreseeable future through better communication and negotiations. Similarly, Plant Variety Rights (PVR) legislation has been claimed to be a major constraint in germplasm exchange (Duvick, 1984; Gill, 1989; Marshall, 1989). Theoretically, cultivars protected by PVR should be freely available for use for breeding and research. In practice, however, breeders in countries with PVR are reluctant to send materials to countries without such legislation due to the lack of legal restrictions preventing the large scale seed

multiplication of a variety and its re-export to other countries. In general, they require that the recipient be an individual who is prepared to guarantee that the material will not be used commercially or provided to third parties without their specific consent.

In the same manner, there are a variety of technical problems, which are commonly regarded as significant limitations to the use of germplasm collections. Among these limitations, the first one is a curator related constraint (Frankel, 1989). Since many collections are relatively new, curators vary markedly in their training and management experience besides their different priorities and attitudes of supplying samples to others. In such ways curators can be the cause of the non-availability of germplasm. Therefore, emphasis needs to be given in improving the technical skills in areas of seed testing, quarantine requirements and procedures, and computer information storage and retrieval systems. A second technical problem that was found in limiting germplasm exchange has been a lack of specificity in requests made by users to curators (Marshall, 1989). Very general or vague requests for valuable materials pose problems especially when the requesters are unknown to the curators and when they are not confident the material will be used to good purpose.

The lack of documentation and description of materials held in germplasm banks is a third major technical problem limiting the wide use of collections (Duvick, 1984; Gill, 1989). When materials have been simply collected and stored in gene banks without full passport data and proper characterization to rescue it from immediate the threat of extinction, such problems occur. Thus, the documentation of collection needs especial emphasis for research funding and training. The other problem is the dissemination of this information once it is collected as they have limited distribution to the potential users. Such constraints are noted to be a major problem in the minor crops, like linseed that do not have international centres.

In the above discussion, some important constraints limiting the full and free availability of germplasm were presented. Below are considered some of the major factors that limit the use of germplasm, even when the materials are available. The most serious problem reported to restrict the use of available germplasm is a lack of plant breeders (Marshall, 1989). This is particularly true in developing countries,

where the number of plant breeders is too low to handle the available crops. Hence, for developing countries, the most important prerequisite to the exploitation of genetic resources for the benefit of their agriculture is the recruiting, training and organization of a body of plant breeders and associated scientific specialists (Marshall, 1989). Likewise, lack of evaluation of collections is widely seen as a major impediment to their utilization (Frankel and Brown, 1984), especially by breeders themselves. That is to say evaluation is a necessary prerequisite to utilization and the more information is available on a collection, the greater its value to the potential users (Clawson, 1985). This also justifies the worthiness of this study on the genetic diversity of linseed.

The other factor noted to limit the utilization of available germplasm by breeders is a lack of pre-breeding (Marshall, 1989). This means the extraction of desirable traits; such as disease resistance or drought tolerance, from non-adapted, unimproved or wild donors and their transfer into high yielding, adapted and improved backgrounds. The derived lines can then be used by breeders in crosses with other elite adapted lines, with the knowledge that they have a high probability of being able to select from such crosses commercially acceptable cultivars carrying the desirable traits.

In summary, breeders and other users can group the constraints that may limit utilization of collections into two broad categories, those that limit the availability of gene banks to users and those that limit the use of readily available materials. In the first category, many factors that are attributable to deficiencies in the management of the collections are included. They can be rectified by providing better scientific training of curators and improved management procedures (Chang, 1984; Marshall, 1989). The latter point include better information flow so that potential users can identify appropriate collections and accessions. It also includes adequate financial support for seed multiplication and sample preparation, packaging and distribution. Similarly, the constraints in the second category are principally user related. Thus, they can be rectified by users and in collaboration with the curators. Generally, the utilization of germplasm is believed to increase when the collections are thoroughly evaluated so that useful variability can be incorporated into high yielding and widely adapted cultivars (Jensen, 1988; Gill,

1989). Therefore, this area of utilizations needs to be adequately addressed to bring about meaningful success and impact especially in developing countries like Ethiopia.

2.10 Drawbacks of limited genetic diversity

Recent advances in plant breeding and the rapid diffusion of improved varieties within a short period of time have reduced the number of main cultivars in major crops (Chang, 1984; Clawson, 1985; McNaught, 1988; Eigenbrode, 1996). Such a small group of cultivars are not only uniform in respect of many agronomic and quality traits but also genetically related or similar. This rapid reduction of genetic diversity and the subsequent result of genetic uniformity lead to what is known as genetic vulnerability (Hammons, 1976; Duvick, 1984; Marshall, 1989). It refers to the risk incurred when large areas are planted with the same crop variety or with varieties having the same genes or cytoplasmic factors that could make the crop vulnerable or susceptible to serious disease and insect ravages, and also to unfavorable environmental conditions (Eigenbrode, 1996).

The uniformity of cultivars, which plant breeders strive for because of market demands, can produce catastrophic effects (Marshall, 1989; Rajaram and Dubin, 1977). This was the key lesson of the southern corn blight epidemic in 1970 in the USA. Since the late 1950s, the Texas cytoplasm was high and made use of the production of maize hybrids, due to its great environmental stability (Day, 1973; Eigenbrode, 1996). In 1960 it had been introduced into 75-90% of commercial maize hybrids. The pathogen of corn blight, which was known to have limited importance, found in this cytoplasm a suitable background for mass propagation. In 1970 an epidemic outbreak of the disease occurred and resulted in a yield loss of 50% in some southern states and 15% in the entire US. Since then, the genetic vulnerability of crop plants favored by uniform varieties is a well-established fact. Similarly, other major crops in the USA exhibited considerable uniformity (Webster, 1976; Hammons, 1976; Kuckuck *et al.*, 1991).

Historically, there have been other disease epidemics that can be traced back to the genetic uniformity of crop species (Kuckuck *et al.*, 1991). The blight disease of potatoes in Ireland in 1846, which resulted in widespread starvation, is probably the

most well known example of all epidemics (Day, 1973; Gemechu and Adugna, 2001). Furthermore, the *Phylloxa* disease in French grapes caused by root aphids around 1850 should also be mentioned (Eigenbrode, 1996). The cultivars of these species are clones and are, therefore, genetically uniform. With all of these crops, the objective was to minimize uniformity by introducing resistant cross parents or, in the case of maize, by incorporating alien cytoplasm for the production of male-sterile cross parents (i.e. to broaden their genetic diversity). The parental diversity or genetic divergence of cross parents must be complemented by regional diversity, incorporating genetic differences within and between crop plants in one region.

The development of high-yielding varieties of crops has greatly benefited some segments of society, but some risks are incurred by use of these new varieties. With the advent of modern plant breeding techniques, a trend toward genetically more uniform agriculture has developed in areas suited to the high-yielding, high-input modern varieties (Marshall, 1989). Whereas traditional mixed farming systems produce modest but reliable yields (Altieri, 1985), planting a single modern crop variety over a large area can result in high yields but the crop may be extremely vulnerable to pests, disease and severe weather (Altieri, 1987). As already mentioned above, the risks of high yielding uniform crops were exemplified by the U.S. corn that suffered a 15 percent reduction in yield and losses worth roughly one billion dollars, when a leaf fungus (*Helminthosporim maydis*) spread rapidly through the genetically uniform crop. Similarly, the Irish potato famine in 1846, the loss of a large portion of the Soviet wheat crop to cold weather in 1972, and the citrus canker outbreak in Florida in 1984 all stemmed from reductions in genetic diversity (Day, 1973; Marshall, 1989; de Boef *et al.*, 1996).

To stabilize production, breeders use one of several tactics to maintain a genetically diverse crop array. Typical varieties are replaced with higher-yielding relatives after four to 10 years even if they still resist disease or pests. In effect, the spatial diversity of traditional agriculture is replaced with a temporal diversity created by a continuous supply of new cultivars (Altieri and Anderson, 1986). In the United States, the average lifetime of a cultivar of cotton, soybean, wheat, maize, oats, or sorghum is between five and nine years (Hawtin *et al.*, 1997). Newer strategies for stabilizing production involve the use of varietal blends (a mix of strains sharing

similar traits but based on different parents) or multilines (varieties containing several different sources of resistance) (Altieri and Anderson, 1986). In each case, the crop represents a genetically diverse array that can better withstand disease and pests. Despite these efforts, genetic uniformity still places some crops at risk of disease outbreaks and in some regions that risk is considerable (Safeeulla; 1977). For example, some 62 percent of rice varieties in Bangladesh, 74 percent in Indonesia, and 75 percent in Sri Lanka are derived from one maternal parent (Chang *et al.*, 1982; Chang, 1984).

2.11 Genetic variability and related issues in linseed

2.11.1 Distribution and utilization

Linseed, which is widely adapted to warm and cool temperate climates, is cultivated for its seed oil. It has traditionally been used for therapeutics because of the laxative properties of mucilaginous carbohydrates (pentosans) associated with the seed coat (Carter, 1993; Aldrich, 1998). Its cultivation was widespread in the Near East region (Israel, Syria, Jordan, Iraq and Iran) in 6000 B.C. (Harlan, 1975; Lay and Dybing, 1989). The ancient Egyptians had a high regard for linen, cloth made from linseed/ flax fiber (Simmonds, 1976; Luhs and Friedt, 1994). They used it not only for clothing but also for the wrapping of mummies (Pizzey, 1998; Mansby *et al.*, 2000). They also embalmed bodies with linseed oil (Simmonds, 1976). Likewise, Roman emperors wore linen garments produced in Babylonia, Greece, Egypt and Spain, whereas the Phoenicians used strong and coarse linen cloth to make snails for their boats (Lay and Dybing, 1989). After flax production spread to Europe, it was grown exclusively for linen until the beginning of 20th century, when cotton took its place. Since flax fibers are stronger, more durable, and more resistant to moisture than cotton or wool, linen fabrics still have superior images (Luhs and Friedt, 1994).

The shorter, branched and early maturing linseed types prefer the warmer climates of Canada, Argentina, India, China and the former USSR, contributing together about 75% of the total 2.65 million tones of linseed produced in 1991 (Luhs and Friedt, 1994). Highest seed yields are generally found from Canada or Argentina that accounts for high overall production (Lay and Dybing, 1989; Flax Council, 2000b). Linseed has about 35-45% oil, which is unique among the major vegetable

oils because of its high level (45-65%) linolenic acid (Green and Marshall, 1981). The high susceptibility to autoxidation gives linseed oil a rapid drying property, which explains the traditional usage in the paint and varnish industry. Raw and cold-processed linseed oil has also been used for nutrition in countries, like India, where about 35-40% of the oil is consumed as cooking oil (Luhs and Friedt, 1994; Flax Council, 2000a). Nevertheless, the high linolenic acid causes rancidity and a short shelf life, and since the commercial production is relatively lower than other oil crops, linseed oil is not commonly used for edible purposes. Attempts are being made to obtain a drastic reduction of the linolenic acid content, which would provide an edible-quality linseed oil. Recently, cultivars with trademarks of 'Linola' and 'Solin' have been patented in Australia and Canada (Rowland, 1994; Flax Council, 2000b). Protein content in linseed ranges from 20 to 24%. After oil extraction, a meal is obtained, which is considered a valuable protein concentrate for livestock (Luhs and Friedt, 1994; Pizzey, 1998).

2.11.2 Taxonomy and general description

The Genus *Linum* belongs to the family *Linaceae*, and is further divided into five taxonomic sections (Luhs and Friedt, 1994) based on chromosome number, cytological studies, from crossing experiments, and flower structure. According to Durrant (1986), the genus *Linum* consists of about 200 species, which are spread over cool and warm temperate regions of the northern hemisphere. *Linum usitatissimum* L., (n = 15) is the only species of agricultural importance, though the closely related (*L. angustifolium*, n = 15) has been formerly cultivated in some areas (Lay and Dybing, 1989). According to these authors, the latter is considered as the most likely progenitor of linseed, but other species such as *L. bienne* Mill may have contributed some germplasm.

Table 2.2. Overall description of linseed plant parts (Seegler, 1983)

Plant part	Overall description
Habit:	annual herb and glabrous, 30-60 cm tall, 2-8 stemmed by basal branching; each stem with 3-7 branches in the upper part, terminating in a flower.
Stems:	erect or ascending, seldom prostrate, cylindrical, up to 6 mm diameter, smooth, pale green, grayish, largest leaves in the middle and less leafy at the apex than basal.
Leaves:	alternate to sub-opposite, sessile, narrowly elliptic to linear or lanceolate, upto 48 mm long and 8 mm wide, dull medium green, trinervat from base almost to the acute top.
Pedicel:	3-22 mm long at anthesis.
Flower bud:	ovoid when full grown, acuminate to acute at apex; contorted petals becoming apparent the day before anthesis.
Sepals:	five, quincuncial, broadly elliptic to ovate, 6-8 mm x 3-4 mm, green, with a transparent, whitish, fimbriate margin, with 3 main veins and 2 smaller ones.
Corolla:	funnel to cup-shaped, soon caducous; petals 5, implanted above receptacle with darker veins, ovate to obtriangular 9-13 mm x 6-11 mm, with bluntly undulate upper margin.
Stamens:	five, alternating with the petals, 0-2 lobes, up to 6.5 mm long; oblong anthers, 1-2 mm long, basifixed, blue or seldom yellow.
Ovary:	ovoid to ellipsoid, 2-3.5 mm diameter, green, 5 epipetalous locules.
Styles:	five, shortly connate at base, filiform, 2-2.5 mm long, white, blue or greenish.
Stigmas:	filiform, 1.5-2 mm long, white, pale blue or greenish.
Fruit:	an indehiscent glabrous boll, broadly ovoid, up to 12 mm high, up to 11 mm diameter, light brown, with up to 10 splits, partially covered persistent calyx, with 5 complete, true septa and 5 sickle-shaped false septa.
Seeds:	up to 10 per fruit, laterally shallowly biconvex, obovate to elliptic in outline, basally with a small beak, 3.75-5.50 mm x 1.5-2.5 mm x 0.75-1.25 mm, usually brown, less often yellowish, glossy and smooth. Embryo large, erect, covered with endosperm. Embryo and endosperm rich in oil
Seedling:	epigeal, with a filiform root system with many laterals. Hypocotyl cylindrical, glabrous, pale green, brownish tinged at base, up to 50 mm long cotyledons opposite, elliptic to obovate, up to 17 mm long, up to 9.5 mm wide, with obtuse. Epicotyl up to 4 mm long and at 5 mm long tillering may start.

The linseed plant is an annual herb with a thin, erect and wiry stem, as summarized in Table 2.2. The cultivars grown for seed purposes are usually smaller, more branched and profusely tillering (Luhs and Friedt, 1994). Small flowers with blue, pink or sometimes white petals grow on terminal panicles. The flowers are highly self-pollinated due to their structure and sticky pollens, which are rarely transferred

by insects (Lay and Dybing, 1989), however cross-pollination may occur at a rate of less than 10% (Luhs and Friedt, 1994). The fruit of linseed is a round boll, divided into five chambers, with each possessing up to two brownish or yellow seeds. Its thousand-seed weight ranges between 3 to 16 grams and is negatively correlated with the number of seeds per boll (Luhs and Friedt, 1994).

2.11.3 Centers of origin and diversity

The cultivation of linseed goes back to the dawn of civilization, as shown by prehistoric Swiss Lake Dwellers (Luhs and Friedt, 1994; Pizzey, 1998; Flax Council, 2000b). There are strong indications that *Linum* originated in an area east of Mediterranean region, notably nearby India (Luhs and Friedt, 1994; Pizzey, 1998), and it spread northwards and westwards Most researchers believe that linseed originated in an area east of the Mediterranean Sea because of the great diversity forms found in this region (Lay and Dybing, 1989). Linseed was grown for its oil and was developed primarily in southwestern Asia, whereas flax or the fiber type was developed in the Mediterranean region (Flax Council, 2000b). Linseed was used as a food during Greek and Roman eras, a practice that has continued in both India and Ethiopia even today (Lay and Dybing, 1989; Adugna, 2000; Flax Council, 2000b). Ethiopia was also reported as a center of diversity (Vavilov, 1951; Harlan, 1969; Mengesha, 1975) for linseed and this makes the current analysis of genetic diversity more worthwhile.

2.11.4 Genetic diversity, heritability and genetic advance

Preliminary study of genetic diversity for linseed in Ethiopia indicated that days to flowering and maturity were in the ranges of 55-130 and 151-178 days, respectively (Getinet *et al.*, 1987). Late maturing and taller plants were collected from higher altitudes of Ethiopia and these plants were susceptible to lodging. Higher ranges of variability were noted for bolls/plant (11-42), seeds/boll (3-14), plant height (17-80 cm) and 1000-seed weight (3.1-5.6 g). Most accessions were observed with small boll sizes, light blue petals and brown seed colour. Among the various diseases of linseed, powdery mildew (*Odium lini*) was reported (Getinet *et al.*, 1987) as the most prevalent especially towards the later stage of plant growth.

Jeswani *et al.* (1970) studied the divergence of 100 linseed accessions, which were obtained from seven geographical regions of the world (Afghanistan, Europe, USSR, India, Australia, North America and South America). Based on Mahalanobis's distance, the Indian group was found distinctly different from the other groups. The European materials were not very diverse from that of the Russian, American and Australian materials. Maximum diversity was found between the Indian and Russian groups followed by the Indian and European groups. Jeswani *et al.* (1970) indicated that the Indian linseed was of polyphyletic origin, which might have been introduced from Central Asia, Egypt and Abyssinian sources. They also reported number of primary branches has contributed maximum to divergence, followed by the height at which fruiting branches starts. Both characters were found as potent factors in differentiating oilseed types from fibre types of linseed.

Another investigation that was carried out on genetic divergence of 40 varieties of linseed revealed wide diversity among the varieties including those from the same source (Asthana and Pandey, 1980). The 40 genotypes were grouped into 12 clusters, six of them with only a single variety each. The clustering pattern also showed that genetic and geographic diversity were not necessarily related. Asthana and Pandey (1980) reported that such a situation might have been caused by frequent exchange of breeding materials from one place to other and its further selection in different geographic regions, which could result in genetic drift. Similar genetic variability of linseed was also studied by Singh (1984) under rainfed conditions at the Ghaghraghat Crop Research Station, Uttar Pradesh of India, and the result revealed that genetic coefficient of variation was highest for bolls/plant, followed by tillers/plant, branches/plant, 500 grain weight, plant height and days to flower. The broad sense heritability was highest for days to flower, yield/plant and plant height. The highest genetic advance was recorded for bolls/plant, though this character showed medium high heritability. The genetic advance showed high value for bolls/plant, tillers/plant and yield/plant. The remaining characters did not show equally high genetic advance in spite of their high heritability (Singh, 1984). High genetic advance (as percentage of mean) accompanied by high or moderate high heritability was recorded for plant height, tillers/plant bolls/plant and yield/plant. Selection was, therefore, reported (Singh, 1984) to be helpful in improving these

characters. However, in order to reach a sound conclusion, heritability, genetic variability and genetic advance should be considered simultaneously.

Morphological variability for some yield components was studied in relation to yield potential in 25 linseed genotypes during 1996-98 at the Podu-Iloaiei Agricultural Research Station, Romania (Ioan, 1998). In most of the studied genotypes, the presence of high phenotypic variability for mean number of bolls per plant and seed weight/plant was noted, the variability coefficient exceeding the threshold of 20%. Thousand seed weight was more stable, shown by lower values of the variability coefficient. One line (L-50240-5-88) was notable for yields of 276 kg/ha in comparison with the control variety. The influence of environmental conditions on yield was noted within the same genotype during the three years of testing. Nichterlein and Friedt (1991) also indicated the influence of both genotypic and environmental factors affecting anther callus regeneration in linseed.

Eighty diverse genotypes of linseed were grown at Rewa during rabi 1995/96 under four artificially created micro-environments (combinations of timely vs. late-sown and irrigated vs. rainfed) and evaluated for yield and its components (Payasi, 2000). The genotypes were grouped into 14 clusters based on D^2 analysis. Eleven genotypes along with the early maturing genotype were identified as diversified genotypes of linseed with potential for future breeding programs. Another field experiment conducted in India (Dayal *et al.*, 1975) revealed a wide variability among 20 linseed varieties. Characters, which were relatively less influenced by environmental conditions, were days to flowering (89% heritability); days to maturity (83%); 1000-seed weight (71%); and plant weight (68%). Additive type of gene action was reported (Dayal *et al.*, 1975) to be operative for these characters.

Variability and character association was studied (Mishra and Yadav, 1999) for eight characters in 72 genotypes of linseed grown at Tikamgarh. Seed yield/plant, bolls/plant and branches/plant had a high genotypic coefficient of variation. High heritability coupled with high genetic advance was observed for seeds/bolls, days to maturity and bolls/plant, indicating the importance of additive gene action for these traits. Number of seeds/plant, bolls/plant, branches/plant and seeds/boll were positively and significantly correlated with seed yield. Seeds/plant and bolls/plant

showed a high positive direct effect on seed yield. It is suggested (Mishra and Yadav, 1999) that selection for seeds/plant and bolls/plant is important to evolve high yield varieties of linseed.

Payasi *et al.* (1999) assessed 80 diverse genotypes of linseed under eight micro-agroclimatic conditions for yield correlations and bolls/plant, secondary branches/plant and seeds/boll. All of them had positive correlations, while days to flower initiation and days to 50% flowering had negative correlations with seed yield/plant. Homogeneity in correlation coefficients of different environments revealed that out of 55 characters combinations 54 were homogeneous but their secondary branches/plant vs. tertiary branches/plant showed significant differences in all environments for two years. Another correlation study (Mahto, 1998) undertaken in 10 characters of linseed showed that yield was strongly and positively correlated with height, number of bolls, number of seeds/boll, number of breakable bolls and biological yield. The genotypes showed wide genetic divergence, substantially contributing to the number of bolls/plant, seed yield and biological yield, which were important characters in correlation (Mahto, 1998). Based on their divergence, two genotypes were recommended to be crossed with each other. In the same manner, Mahto and Verma (1998) evaluated 59 genotypes of linseed for genetic diversity under rainfed and irrigated conditions and they were clustered in 10 groups. Cluster I was biggest and contained 19 genotypes. There was no parallelism between clustering pattern and geographic origin. Maximum genetic diversity was obtained between cluster VI and VIII. This indicated the potential for heterosis in crosses between four genotypes of cluster VI and three genotypes of cluster VIII.

Chandrashekhar-Mahto *et al.* (1998) studied the genetic variability of linseed with 17 diverse genotypes as parents and their hybrids by crossing in a line x tester (15 x 2) mating design for 11 quantitative traits. The genotypic coefficient of variation was high for number of bolls per plant, seed yield per plant, number of secondary branches per plant and 1000-seed weight. High heritability in the narrow-sense was obtained for 1000-seed weight, days to 50% flowering, plant height and days to maturity. Higher heritability along with high genetic advance for these characters indicated predominance of additive gene action in expression of these characters.

Chandrashekhar-Mahto *et al.* (1998) suggested that phenotypic selection on the basis of these traits might be effective for yield improvement.

Mahto and Singh (1998) studied 20 genotypes of linseed at Birsa Agricultural University (India) over four environments (two seasons, irrigated/rainfed). Pooled data of nine characters over environments were analysed for D^2 estimates, and the mean sum of squares for most characters revealed highly significant differences due to genotypes (G), environments (E) and G x E interactions for all characters. Stability of individual genotypes was determined on the basis of two stability parameters (regression coefficient and deviation from regression). Seven genotypes were selected with high seed yield/plant (higher than grand mean over environments) and stability for seed yield and for other yield contributing characters.

Mahto and Mahto (1998) studied the variability, heritability and correlation coefficients for seven yield-related characters in 19 genotypes of linseed grown under rainfed conditions during the winter seasons of 1990/91 and 1994/95. Seed yield/plant, number of bolls/plant, number of secondary branches/plant and primary branches/plant had high genetic coefficients of variation. The highest heritability was given by days to maturity. Seed yield per plant was correlated with days to maturity, plant height, number of primary branches/plant, number of secondary branches/plant and number of bolls/plant at the genotypic level. Path coefficient analysis revealed the highest direct effect on yield of number of primary branches/plant.

Seeds of linseed were harvested (Froment *et al.*, 1998) from sites in Hampshire, Cambridgeshire, Lincolnshire and Aberdeen, dried, threshed and analysed for oil composition using a capillary gas chromatograph. There were significant site x cultivar interactions in the percentages of fatty acids in oils from industrial linseed lines (Froment *et al.*, 1998). The Hampshire site had the highest 1000-seed weights and the Lincolnshire site had the highest yields. Similarly, 30 genotypes were evaluated at Gazipur during rabi 1992/93 for yield and eight of its components. Analysis of variance showed significant differences among the genotypes for all traits except primary branches/plant and seed weight (Mirza *et al.*, 1996). High

phenotypic and genotypic coefficients of variation were observed for yield/plant, harvest index, bolls/plant and plant height. Heritability along with genetic advance as percentage of mean was estimated as high for plant height, harvest index, seed yield/plant and bolls/plant. Harvest index and bolls/plant showed significant positive correlations with yield. Path analysis showed that days to flowering, bolls/plant and harvest index had the highest positive direct effect on yield (Mirza *et al.*, 1996).

A greenhouse experiment with eight linseed cultivars showed that there were significant differences among cultivars for plant height, height of first branch, bolls/plant, seeds/boll, 1000-seed weight, seed yield/plant, harvest index (Kurt, 1996). There were significant positive correlations between harvest index and seed index, between seed yield/plant and height of first branch and bolls/plant, between plant height and height of first branch, and between height of first branch and bolls/plant and seed yield/plant. There were significant negative correlations between 1000-seed weight and height of first upper branch, between number of bolls/plant and seeds/boll, and between seed yield/plant and 1000-seed weight, according to Kurt (1996).

Information on genetic divergence was derived from data on nine yield components in 20 linseed genotypes grown during two winter seasons of 1989 and 1990 at Chota Nagpur, India (Mahto and Singh, 1996). There were significant differences among the genotypes for all the characters, and the genotypes were grouped into three clusters. The greatest inter cluster distance was between clusters I and III. Analysis of estimation of contribution of characters to genetic divergence indicated that number of primary and secondary branches, plant height, bolls/plant, days to 50% flowering, days to maturity and seed yield per plant were significant (Mahto and Singh, 1996). Similarly, information on heritability, genetic advance and yield correlations is derived (Khan and Gupta, 1995) from data on six seed yield-related characters in 26 genotypes grown at R.S. Pura. Path analysis indicated that selection for reduced height and 1000-seed weight would lead to increased yields (Khan and Gupta, 1995).

Mahto (1995) reported on the variability and stability on seven seed yield-related

characters in 19 genotypes grown in three rainfed rabi environments during 1989/90 to 1991/92. Genotype x environment interaction was significant for branches/plant and highly significant for plant height, seeds/boll and bolls/plant. D^2 analysis grouped the 19 genotypes into seven clusters. Twelve genotypes were identified on the basis of stability and genetic divergence for yield and yield attributes. For most of the characters studied three genotypes (BAUL135, LCK8657 and Sweta) were the most stable, according to Mahto (1995).

Agrawal *et al.* (1994) reported that information on yield correlations was derived from data on nine yield components in three linseed varieties grown at Jabalpur during rabi 1990 and 1991. The correlation study revealed that yield was highly and positively correlated with crop biomass/ha, number of bolls and boll weight/plant, number of seeds/plant, plant height, dry weight/plant, branches/plant, test-weight and seed yield (r = 0.99 to 0.89). Regression analysis indicated that the most important characters contributing to seed yield/ha are seed yield/plant followed by number of branches/plant, test-weight, boll weight/plant, harvest index and dry weight/plant. Similar correlation and path analysis of 72 eco-geographically divergent varieties of linseed indicated that seed yield had high positive correlation with bolls/plant and moderate positive correlation with seeds/boll, and significant negative correlation with 1000-seed weight (Muduli and Patnaik, 1994). According to these workers, path coefficient analysis revealed that bolls/plant and seeds/boll were the major components determining yield.

Genetic variability in 17 genotypes and 42 crosses was studied (Verma and Sinha, 1993) under irrigated and rainfed situations. A wide range of phenotypic variation was noted for the important quantitative characters. On the basis of genetic coefficient of variation, heritability and genetic advance, it was concluded that selection based on plant height, 100-seed weight, diameter of boll, days to first flowering and primary branches/plant would be more effective (Verma and Sinha, 1993). Similarly, path analysis for 13 yield and quality influencing characters was conducted on 13 linseed accessions during 1995/96 (Rashid *et al.*, 1998). Seed yield/plot showed positive and significant genotypes correlation with bolls/plant, seeds/boll, 100-seed weight and oil content. The genotypic associations of seeds/boll and 100-seed weight with seed yield/plot were positive and significant

but they had direct negative path towards seed yield/plot. Days to flowering and plant height had negative genotypic correlation with seed yield per plot but they had a positive direct effect towards seed yield/plot. Protein content and oleic acid had negative association and direct negative path towards seed yield/plot. For reliable selection index, bolls/plant and oil content were reported (Rashid *et al.*, 1998) as the characters of prime importance.

Research results were also reported (Cremaschi *et al.*, 1994) from two years' trials (1991-92) of 33 accessions in the Bologna area of Italy. The highest seed yield was obtained from Lidgate variety (3.12 t ha^{-1}). Despite the observed year x variety interaction, it was possible to find some varieties with good yield and high yield stability, such as Adin, Amazon, Azur, Barbara, Blue Chip, BS89C, Buseto Palizzolo, Crystal, Lidgate, Linda, Ocean, Olin and ecotype Camporeale. Regarding to oil yield, BS89C, Adin, Barbara and Lidgate were ranked highest (mean exceeding 1300 kg ha^{-1}). Similarly, field and laboratory studies were conducted (Vasile *et al.*, 1994) on 568 genotypes covering wild species, local populations, cultivars and lines from different collections. Then, some germplasm sources were identified with potential breeding values for earliness, oil content, seed size, and resistance to wilt (*Fusarium oxysporum* f.sp. lini) and to powdery mildew (*Oidium* spp.), according to Vasile *et al.* (1994).

A recent study of genetic diversity in Swedish flax showed that total genetic diversity for the evaluated 18 accessions was very high, and genetic diversity within accession was even the highest (Mansby *et al.*, 2000). This highest genetic diversity within accessions was attributed to greater out-crossing rates occurred under their testing conditions. Another recent study conducted (Diederrichsen, 2001) on the comparison of flax genetic diversity between Canadian cultivars and world collection, indicated that the Canadian cultivars had more range of diversity for fatty acid compositions. The world collection also possessed considerable diversity in economically important characters, such as seed weight, oil content and plant height. Diederrichsen (2001) also suggested that more emphasis should be given to the study of genotypic diversity through the heritability of these traits rather than via phenotypic variations, which have too large environmental influences to express these characters.

CHAPTER 3

DIVERSITY ANALYSIS OF LINSEED ACCESSIONS UNDER GLASSHOUSE CONDITIONS IN SOUTH AFRICA

3.1 Abstract

Sixty linseed accessions predominantly from Ethiopia were evaluated for 11 quantitative characters in a three times replicated randomised complete block design under glasshouse conditions at the University of Free State, South Africa during 2000 and 2001. The major objectives were to assess the diversity of the accessions, and to estimate broad sense heritability and genetic advance of various characters. The mean squares of the accessions were highly significant ($P < 0.01$) for almost all characters. The phenotypic coefficient of variation (CV) ranged from 1.90% for seeds/boll to 142.72% for plant height, while the genotypic CV varied between 0.52% and 118.88% for the same characters. Estimates of heritability ranged from about 15.60% for seed yield/plant to 85.82% for initial days to flowering. Values of predicted genetic gains (as percent of mean) varied from about 3.16% for days to maturity to about 24.26% for plant height. Among the 10 principal components involved in explaining the entire variation between the collections, the first five had eigenvalues of 66.43% of the total variance. The first principal components, which accounted for about 20% of the entire variability was due to days to flowering, maturity and seeds/boll. Cluster analysis grouped the 60 accessions into 11 major classes consisting of one to 24 genotypes. Hence, this study demonstrates the existence of high diversity among the evaluated germplasm of linseed that should be maintained and utilised in future improvement and development efforts to ensure continued survival.

3.2 Introduction

Linseed (*Linum usitatissimum* L.) has been cultivated globally for two primary purposes, seed and oil yields. However, the long-stemmed flax types have also been used for fibre production since the ancient days of Egyptians and Romans (Lay and Dybing, 1989;

Mansby *et al.*, 2000). In Ethiopia, linseed has traditionally been used for food and as a cash crop since antiquity (Seegler, 1983; Adugna and Labuschagne, 2002).

Collection and characterisation of linseed has been undertaken in Ethiopia at Holeta Research Centre since the early 1980s (Getinet *et al.*, 1987) in collaboration with the Plant Genetic Resources of Ethiopia (now Institute of Biodiversity Conservation and Research, IBCR). Consequently, about 641 collections were reported available in the IBCR (Abebe *et al.*, 1992). In the year 1981, 130 accessions were collected from eight administrative regions of Ethiopia (Getinet *et al.*, 1987), and a year later, 129 accessions were characterized at Holeta for 15 traits. However, only descriptive statistics (range, mean, standard deviation and frequency distribution of major traits) were employed to analyse the accessions. These preliminary characterization activities were continued, on a limited scale, to select and advance elite materials, besides rejuvenating the seeds. However, systematic and in depth studies were not conducted to generate the level of information that is required by the current and future breeding programs.

Although Ethiopia is the centre of diversity for linseed and the crop has been under cultivation for long periods of time (Seegler, 1983; Adugna and Adefris, 1995), no significant efforts have been done towards the collection and utilization of its germplasm. Its genetic diversity and eco-geographic pattern of variability were not studied across environments to make use of the important genes. Moreover, the wide range of agro-climatic conditions of the country (MoA, 1998; Adugna, 2000) may have continued contributing to the diversity of this crop. Only limited efforts were undertaken to collect and utilize the germplasm to their full potential. In a nutshell, information on the extent of genetic diversity of linseed in Ethiopia is very meagre and this study was undertaken to address these issues. Hence, the specific objectives of this study were: a) to assess the diversity of linseed accessions for agronomically useful traits; b) to quantify the extent of phenotypic and genotypic diversity among these accessions; c) to estimate heritability (broad sense) and predicted genetic gains of the traits, and d) to identify and cluster desirable groups that could be utilized in the breeding programs. The premise is, therefore, that by analysing and broadening the

germplam basis, more productive genotypes could be developed in a sustainable manner, and this could contribute positively to the strengthening of future improvement and conservation efforts. In other words, the development of comprehensive, well documented and accessible germplasm is essential for the long-term programs of plant breeding so that appropriate varieties can be identified and manipulated to improve low yield and other qualities of linseed.

3.3 Materials and Methods

Plant materials

The 60 accessions used in this study were selectively taken from the Highland Oil Crops Research Program of Holeta Research Centre in the Ethiopian Agricultural Research Organization to represent the apparent diversity of linseed germplasm in the country. The accessions have been collected by the Plant Genetic Resource Centre of Ethiopia (now Institute of Biodiversity Conservation and Research, IBCR) in collaboration with the Highland Oil Crops Research Program since the early 1970s. The germplasm collections are preserved at the IBCR and some of their duplicates are also kept at Holeta for research purposes. Totally 60 accessions, including four released varieties (Chilalo, CI-1525, CI-1652 and Belay 96) were studied for their variability and genetic divergence. Belay 96 was developed and released in 1996 from a crossing program undertaken between locally well-adapted and disease resistant collections at Holeta Research Centre. Fifty-three accessions were collected from different areas of Ethiopia and Eritrea (Figure 3.1), the remaining seven being introductions from Europe, USA and Canada (Table 3.1).

Table 3.1. List of the studied linseed accessions along with their collection areas

No.	Accession	Collection area/ district	No.	Accession	Collection area/ district
1	10002	Shewa/ Ambo	31	10169	Gonder/ Chera
2	10005	Sidamo/ Bore	32	10176	Gojam/ Guangua
3	10007	Kefa/ Omonada	33	10179	Shewa/ Alem-Gena
4	10008	Gonder/ Fogera	34	10185	Shewa/ Chelia
5	10010	Hararge/ Chiro	35	10192	Shewa/ Dendi
6	10022	Welo/ Kalu	36	10197	Shewa/ Ambo
7	10026	Gojam/ Dambecha	37	10204	Welo/ Bati
8	10037	Sidamo/ Bore	38	10006	Ilubabor/ Gore
9	10039	Eritrea/ Mendefera	39	10042	Tigray/ Lay-Machew
10	Chilalo	Arsi/ n.a.	40	Belay 96	Shewa/ Holeta
11	10041	Bale/ Adaba	41	10047	Ilubabor/ Bedele
12	10046	Welega/ Bedele	42	10062	Bale/ Robe
13	10060	Gonder/ Chera	43	10235	Gonder/ n.a.
14	10061	Gamo-Gofa/ Mirab-Abay	44	10236	Gonder/ n.a.
15	10068	Gojam/ Bahir-Dar	45	10064	Gamo-Gofa/BakoGazer
16	10080	Hararge/ Habru	46	10072	Gojam/ Dejen
17	10085	Hararge/ Kuni	47	10073	Gojam/ Maychekel
18	10104	Shewa/ Dendi	48	10246	Ethiopia/ n.a.
19	10109	Shewa/ Tach-Bet	49	10248	Ethiopia/ n.a.
20	CI-1525	Europe/ n.a.	50	10250	Ethiopia/ n.a.
21	10111	Shewa/ n.a.	51	10252	Ethiopia/ n.a.
22	10118	Shewa/ Ambo	52	10254	Ethiopia/ n.a.
23	10119	Shewa/ Chelia	53	10256	Ethiopia/ n.a.
24	10120	Sidamo/ Adola	54	10258	Ethiopia/ n.a.
25	10125	Tigray/ Inderta	55	10260	Ethiopia/ n.a.
26	10138	Welega/ Abe-Dongoro	56	Omega	USA/ North Dakota
27	10144	Welo/ Dese-Zuria	57	N1266	Canada/ Saskatoon
28	10159	Gonder/ Dabat	58	M20G	Canada/ Saskatoon
29	10162	Gonder/ Dabat	59	CDC-1747	Canada/ Saskatoon
30	CI-1652	Europe/ n.a.	60	CDC-VG	Canada/ Saskatoon

Note: n.a. = Information not available on specific collection areas or districts

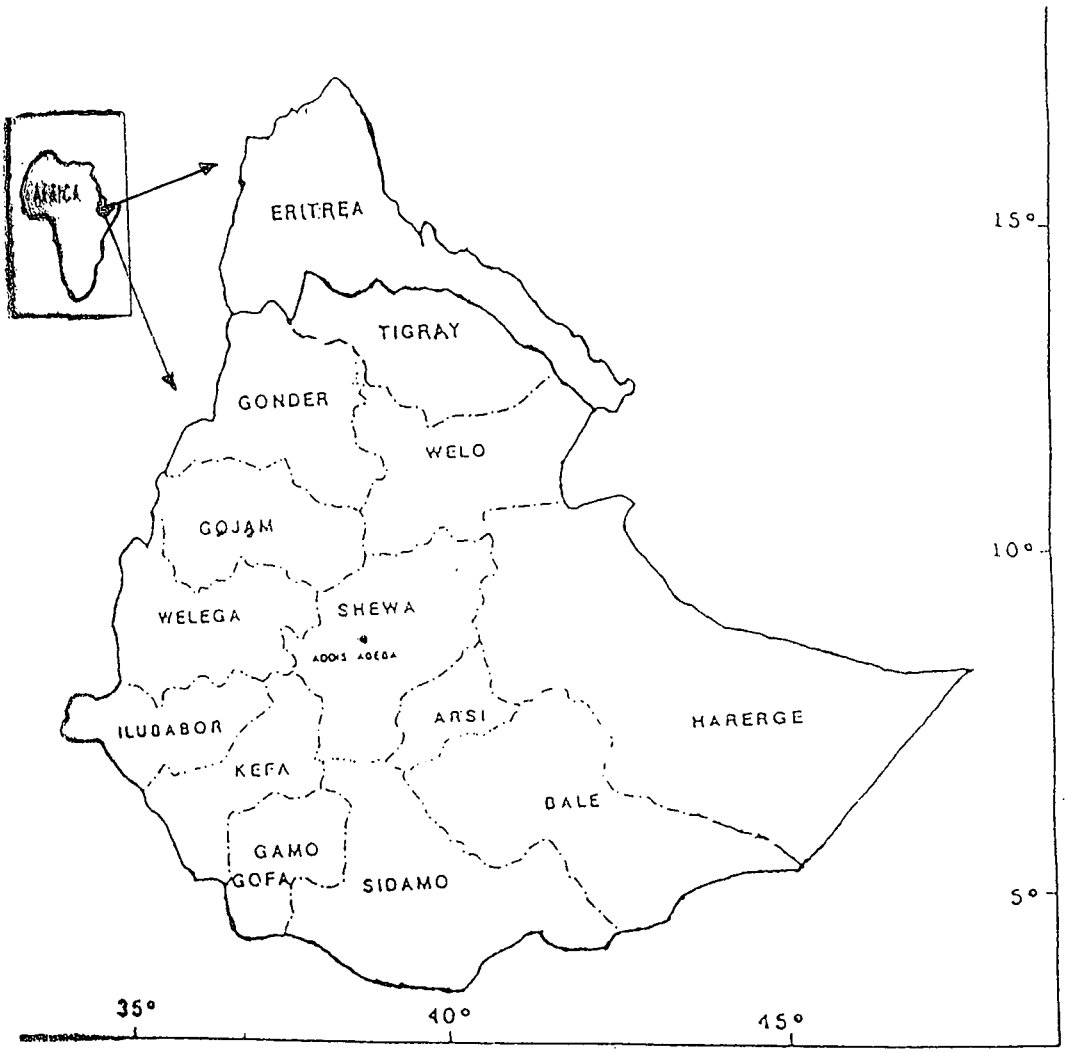


Fig. 3.1. Map of Ethiopia and Eritrea depicting regions from where the linseed accessions were collected. (Solid lines represent international boundaries, while broken lines show boundaries between previous provinces within Ethiopia)

Experimental environment and methods

The study was conducted in one glasshouse during the year 2000 and in another one in 2001 at the University of the Free State (latitude 29° 6' S, longitude 26° 18' E, altitude 1351 m above sea level) in accordance with the availability of working spaces. The former glasshouse was warmer by about 10°C (average) than the latter, which had a maximum temperature of up to 25°C during the daytime. The minimum night temperature was in the range of 8 to 13°C. Daylight hours ranged from 10.1 in June to 13.9 in December, and April to September had relatively shorter 11.3-11.8 hours, of daylight. The 60 accessions were evaluated in pots (2.5 litres) that were laid out in a randomised complete block design with three replications. Eight seeds were sown at a depth of about 2.5 cm and thinned to five plants per pot three weeks after planting. The seeds were sown in early September during the first year and in mid June during the second year. A mixture of nitrogen and phosphorus fertilizers of 3 g per pot was applied to the red sandy soil of the Free State province at sowing, 50% flowering and seed filling stages. The plants were irrigated almost every day from planting to maturity.

Data on the agronomical and morphological characters (Diederichsen, 2001) were collected from three randomly selected plants and their means were recoded for all observations. Except the days to initial flowering and 50% flowering, all the remaining data were recorded at maturity and thereafter. Days to initial flowering represent the days from planting to the appearance of the first flower/s, whereas days to flowering and maturity stood for the number of days from sowing to 50% anthesis and ripening, respectively. The unit of measurement for plant height was in centimetres (cm), while that of seed yield and 1000-seed weight was in grams (g). In general, evaluation was undertaken based on the major phenological and morphological quantitative descriptors that are reported to display highly heritable characters (Diederichsen, 2001).

Statistical analysis

The mean values of all sampled observations for the 11 quantitative characters were analysed for their variance and significance using AGROBASE 98 software (Agronomix Software, Inc., 1998). The critical difference (CD) and coefficient of variation were also calculated for comparing means of the accessions and to see the precision levels. Average of data over the two years were used in defining some basic statistic, such as range and mean. The same mean data were also used for the analyses of principal components and clustering by which classification of the accessions was investigated. In employing the analyses of both principal components and clusters, the 60 accessions were considered as operational taxonomic units and they were represented by rows and 11 characters taken as variables by columns. Both the principal component and cluster analyses were performed by using the Number Cruncher Statistical System, NCSS 2000 (Hintze, 1998) statistical system for Windows. In the process of hierarchical clustering, unweighted pair group method of arithmetic average (UPGMA), Manhatan distance and standard deviation scaling type were employed.

Estimation of genetic parameters were done to identify and ascertain the genetic variability among accessions and to determine the presence of environmental effects on various characters. The variance components were based on the combined analyses over the two years and they were used in partitioning the gross (phenotypic) variability into its components due to genotypic (hereditary) and nonhereditary (environmental) factors, as indicated by Kebebew *et al.* (1999). Moreover, these genetic parameters were estimated by adapting the formulae suggested by Allard (1960), Singh and Chaudhary (1977), Fehr (1987), Kebebew *et al.* (1999) and Atlin *et al.* (2001). Accordingly, genotypic and phenotypic components of variance and their coefficients of variability were estimated by applying the following formulae: (i) Genotypic variance, GV = MSa - (MSay/y - MSe/ry), where MSa = Mean square of accessions, MSay = Mean square of accession x year interaction; MSe = Mean square of error, r = number of replications; y = number of years (ii) Phenotypic variance, PV = GV + (MSay/y + MSe/ry); (iii) Genotypic coefficient of variability, GCV% = (GV/grand

mean) x 100; (iv) Phenotypic coefficient of variability, PCV% = (PV/grand mean) x 100; (v) Heritability (broad sense) for all the characters were computed as H^2 = GV/PV x 100; (vi) Genetic advance, GA = H^2 x PS x K, where PS = phenotypic standard deviation ($\sqrt{PV}$), and K is selection differential at 5 percent selection intensity = 2.06; GA% (as % of mean) = GA/grand mean x 100.

3.4 Results

The mean squares of combined analysis of variance over the two years showed highly significant ($P < 0.01$) differences between years, accessions and their interactions for most of the 11 characters, indicating substantial variability among the tested accessions. The seasonal variability and its interaction with the accession were mainly due to differences in temperature of the two glasshouses, as mentioned in the materials and methods section. The variability among the accessions was also confirmed by the wide range of differences observed between the minimum and maximum values of the 11 quantitative traits (Table 3.3). For example, plant height varied from 52 to 94 cm, while days to maturity ranged between 123 and 148 days. Similarly, seed yield was in the range of 62 to 158 g/m^2, while seed yield per plant varied from 1.67 to 3.02 g. The highest seed yield was obtained from Belay 96, one of the commercial varieties released in 1996 from Holeta Research Centre. Other traits, like number of primary and secondary branches, bolls per plant and seeds per boll also revealed a wide range of variation, as shown in Table 3.3 along with their grand means and critical differences. If the difference of any two accessions is greater than the given critical differences, the variability between the accessions is considered significant (Singh and Chaudhary, 1977). In general, the large differences between the minimum and maximum values of all 11 traits and the highly significant ($P < 0.01$) differences between the accessions indicate the presence of tremendous variability among the studied accessions of linseed.

The estimated values of phenotypic variance were in the range of 0.08 for seed yield per plant to 101.90 for plant height (Table 3.4). Likewise, the lowest genotypic variance of 0.01 and the highest (84.88) were found for the same characters of seed

yield per plant and plant height, respectively. The next largest genetic variances were recorded for days to 50% flowering (36.09), initial days to flowering (24.95) and days to maturity (11.08). These variances demonstrated the existence of large variability for most of these studied characters. Moreover, the phenotypic coefficient of variation ranged from 1.90% for seeds/boll to 142.72% for plant height. Genotypic coefficient of variation was also lowest (0.52%) for seed yield/plant and highest (118.88%) for plant height. The observed higher percentages of both phenotypic and genotypic values were due to their respective larger variances and lower means (Table 3.4). In general, traits that had relatively larger genetic variances also showed higher genotypic coefficients of variation, suggesting that selection for these characters could be more effective than the remaining ones since they had less environmental influences.

The estimated broad sense heritability values for the 11 characters varied from 15.60 to 85.82%. The lowest value was recorded for seed yield per plant, while the highest was for initial days to flowering. Characters, like days to 50% flowering, plant height, seeds/boll and secondary branches/plant also exhibited a relatively higher heritability (73-85%) than other characters, such as seed yield per plant and seed yield per unit area (Table 3.4). This suggests that selecting for traits with high heritability could lead to better progress than selecting for yield/plant and yield (g/m^2) that had relatively lower heritability, as they were more influenced by the environment. Similar results were also reported (Kebebew *et al.*, 1999) in tef [*Eragrostis tef* (Zucc.) Trotter] and other cereal crops. Generally speaking, for characters with lower heritability levels, for instance yield/plant and yield (g/m^2), selection may be difficult due to substantial effects of the growing environments. Thus, broad sense heritability would be more reliable if accompanied by a high genetic advance, as suggested elsewhere (Geleta, 1998; Kebebew *et al.*, 1999).

Genetic gains that could be expected from selecting the top 5% of the genotypes, as a percent of the mean, varied from 3.16% for days to maturity to 24.26% for plant height (Table 3.4). Relatively higher genetic advances are expected from plant height, secondary branches/plant, bolls/plant and days to 50% flowering, and an increase of

14-24% could be made by selections based on these traits under similar environmental conditions to the two glasshouses used. Larger genotypic coefficients of variation along with high heritability and high genetic advance were reported (Geleta, 1998; Kebebew *et al.*, 1999) to provide better information than a single parameter alone. Therefore, characters that exhibited a high genotypic coefficient of variation, heritability and genetic advance would be very useful as a base for selection. Comparatively, larger values of these three parameters were recorded for plant height, bolls/plant, days to 50% flowering and secondary branches/plant.

Table 3.2. Mean squares and other measurements of the combined analysis of variance for 11 quantitative traits of 60 accessions of linseed evaluated in two glasshouses at University of the Free State (UFS) in South Africa, 2000-2001

Source	df	Characters										
		PH	IDF	DF	DM	PB/P	SB/P	B/P	S/B	SY/P	TSY	TSW
Year (Y)	1	117470**	61518**	90884**	151906**	5.63**	34.23**	996.67**	0.07	0.01	0.25	575.88**
Reps (Y)	4	2526.19	17.05	40.31	62.23	5.48	56.69	542.20	0.99	0.07	26.79	159.52
Entry (E)	59	611.40**	174.46**	253.48**	170.78**	0.66**	4.07**	37.49**	1.07**	0.47*	11.85*	2.99**
Y x E	59	187.11**	36.09**	50.94**	152.01**	0.38	0.58	21.50	0.01	0.55*	13.74**	2.30
Residual	236	59.65	19.07	29.93	80.43	0.39	1.33	20.55	0.31	0.32	8.02	1.83
Range		52-94	57-82	66-96	123-148	3-5	4-9	7-20	8-10	1.67-3.0	62-158	7-10
R^2 (%)		92.54	94.27	93.91	90.02	48.82	63.06	57.81	48.30	48.06	46.07	77.97
CV%		10.84	6.41	6.96	6.64	15.12	19.72	13.74	5.90	18.67	23.97	16.67

Note: *, ** = Significant at $P < 0.05$, 0.01, respectively; PH = Plant height at maturity; IDF = Initial days of flowering; DF = Days to 50% flowering; DM = Days to maturity; PB/P = Primary branches/plant; SB/P = Secondary branches/plant; B/P = Bolls/plant; S/B = Seeds/Boll; SY/P = Seed yield/plant; TSY = Total seed yield (g/m^2); TSW = Thousand seed weight; Reps = Replications; R^2 = Repeatability; CV = Coefficient of variation; df = degree of freedom

Table 3.3. Mean performance of the measured traits for 60 linseed accessions evaluated in glasshouses at UFS (South Africa), 2000-2001

	Trait										
Accession	PH	IDF	DF	DM	PB/P	SB/P	B/P	S/B	SY/P	TSY	TSW
1. 10002	66	71	86	138	4	6	14	9	2.56	97.00	7.49
2. 10005	57	65	77	140	4	6	15	10	2.05	91.50	7.23
3. 10007	86	82	95	141	5	7	17	10	2.53	99.00	7.31
4. 10008	71	79	91	137	4	6	14	10	2.30	100.50	7.67
5. 10010	65	64	78	138	4	6	12	9	2.68	109.00	7.18
6. 10022	94	71	85	148	5	5	11	9	2.36	87.00	8.05
7. 10026	87	72	83	131	5	6	14	9	2.63	102.50	8.28
8. 10037	76	64	75	139	5	7	17	9	2.22	104.00	6.97
9. 10039	72	63	72	130	4	6	12	9	2.74	113.50	9.74
10. Chilalo	88	71	78	133	4	5	14	9	2.74	134.50	8.75
11. 10041	77	64	73	134	4	5	14	9	2.12	90.00	9.17
12. 10046	62	60	72	134	4	5	12	10	1.87	81.00	8.31
13. 10060	75	71	85	144	4	6	14	9	1.80	85.00	8.93
14. 10061	70	71	80	136	4	7	14	10	1.91	92.50	7.40
15. 10068	70	69	79	130	4	5	12	10	2.28	112.50	8.18
16. 10080	59	62	70	124	5	6	14	10	2.56	73.50	8.28
17. 10085	64	69	79	136	4	7	13	9	2.55	83.50	7.98
18. 10104	65	70	80	140	4	6	14	10	2.16	101.50	8.72
19. 10109	63	58	72	131	4	6	12	9	2.01	88.50	7.66
20. CI-1525	90	71	78	143	4	7	12	9	2.52	138.50	9.62
21. 10111	70	72	85	141	4	5	21	10	2.29	123.50	8.30
22. 10118	72	71	80	143	5	6	12	10	2.57	116.00	8.27
23. 10119	72	70	87	141	4	6	13	10	2.20	111.00	8.33
24. 10120	81	63	71	129	4	7	16	9	2.63	107.50	8.21
25. 10125	54	63	73	133	4	5	13	9	1.96	79.00	8.25
26. 10138	65	72	84	132	4	6	14	10	2.33	102.00	7.19
27. 10144	69	63	71	130	4	6	12	9	2.54	113.00	7.99
28. 10159	67	60	67	136	4	5	12	9	2.41	104.50	7.91
29. 10162	63	59	67	127	4	5	11	9	2.69	105.50	8.39
30. CI-1652	82	71	80	140	4	6	13	9	2.45	142.50	9.15
31. 10169	74	73	83	136	4	5	9	10	2.41	116.00	7.88
32. 10176	65	71	84	139	4	5	9	9	1.98	103.00	6.98
33. 10179	64	68	81	136	4	7	12	9	2.18	105.50	7.53
34. 10185	76	71	81	133	4	6	13	10	2.48	103.00	8.62
35. 10192	67	70	85	135	5	5	13	10	2.22	112.00	7.80
36. 10197	70	73	85	140	4	5	10	10	2.15	126.50	7.87
37. 10204	78	81	96	147	4	5	8	9	1.67	62.00	6.95
38. 10006	57	58	66	123	5	7	18	9	2.96	109.50	7.57
39. 10042	73	64	73	136	4	6	14	10	2.63	109.00	7.61
40. Belay 96	86	70	79	136	4	6	15	10	3.02	158.00	7.58
41. 10047	63	61	72	130	4	5	16	9	2.42	79.50	9.25
42. 10062	82	63	73	137	4	6	11	8	2.06	94.50	8.70
43. 10235	68	68	77	134	4	5	12	10	2.30	93.00	8.09
44. 10236	55	57	67	123	4	5	11	8	2.55	82.50	8.30
45. 10064	52	64	75	126	4	5	11	10	2.36	75.50	7.41
46. 10072	58	70	80	136	4	5	12	9	1.93	70.00	7.53
47. 10073	67	68	76	127	4	6	17	10	2.52	91.50	7.62
48. 10246	72	65	75	133	4	6	14	9	2.46	65.50	8.33
49. 10248	65	72	80	135	4	5	16	9	2.22	89.50	8.44
50. 10250	65	73	80	140	5	6	16	9	2.36	84.50	7.32
51. 10252	66	71	82	136	4	5	14	10	2.83	108.50	8.08
52. 10254	68	74	85	136	4	5	12	10	2.26	105.50	8.14
53. 10256	66	69	78	134	5	6	12	10	2.47	104.50	7.86
54. 10258	66	69	84	136	5	5	15	9	2.43	113.00	8.21
55. 10260	83	77	90	138	4	6	14	10	2.42	78.50	8.54
56. Omega	86	69	76	132	5	7	17	9	2.28	129.50	8.39
57. N1266	88	68	75	130	5	7	18	9	2.28	107.50	9.10
58. M20G	92	73	80	137	5	7	19	9	2.90	137.50	9.84
59.CDC1747	81	67	75	139	4	8	20	10	2.11	106.00	6.87
60.CDCVG	79	66	76	134	4	6	14	9	2.43	143.50	9.14
Mean	71.40	68.23	78.70	135.2	4.23	5.84	13.67	9.40	2.33	102.53	8.11
CD 0.05	10.46	5.91	7.41	12.14	0.85	1.56	6.14	0.75	0.66	12.85	1.83

Note: Please refer Table 3.2 for the abbreviations

The principal component analysis grouped the 11 variables into 10 components, which accounted for the entire (100%) of the variability evident among the tested accessions. It also revealed that the first nine eigenvectors explained 99.53% of the total variance. Of these, the first five (the ones with eigenvalues greater than one) accounted for a cumulative value of about 66.43%, while the first three accounted for about 48% of the total variability among the accessions (Table 3.5). The first principal component (PC) which alone explained about 20% of the entire variability among the collections was due mainly to differences in days to flowering and maturity, and number of seeds/boll (Table 3.5). Similarly, about 19% of the total variability of the test genotypes accounted for by the second PC originated primarily from variations in plant traits including plant height, bolls/plant, initial days to flowering, primary branches/plant, seed yield/plant and seed yield (g/m^2). The third PC, which accounted for 9.3% of the variation among the accessions, resulted largely from variability in 1000-seed weight, plant height, and days to maturity. The PCs from the 4^{th} to 7^{th} each accounted about 9% of the variation among the accessions arising from different plant characters (Table 3.5).

Cluster analysis based on the 11 characters showed that the 60 accessions were classified into 11 groups (Table 3.6 and Fig. 3.2). Cluster I and III each had three accessions, while cluster II and IV consisted of 23 and 24 accessions, respectively. The remaining seven clusters had one accession each, indicating their wide variability from the others. The accessions were not necessarily grouped according to their geographic origin or specific collection sites. For example, cluster I was composed of one accession from Ethiopia collected from Sidamo area, one from the USA and the other from Canada. The same was true with cluster IV, which had collections from different regions of Ethiopia, Eritrea, Europe and Canada. Clusters II and III consisted of accessions from Ethiopia, but from different areas within Ethiopia. Nevertheless, the clustering process did not necessarily reflect the geographic origins of the collections.

Accessions in cluster I were generally characterized by lower 1000-seed weight, more bolls/plant, intermediate maturing and fairly high yielding plants (Table 3.7). Cluster II that consisted of 23 accessions had a common feature of shorter plants, early maturing and more number of seeds/boll.

Table 3.4. Estimates of phenotypic and genotypic parameters for 11 measured traits of 60 linseed accessions studied in glasshouses at the UFS (South Africa), 2000-2001

Genetic parameter	Characters										
	PH	IDF	DF	DM	PB/P	SB/P	B/P	S/B	SY/P	TSY	TSW
Mean	71.40	68.23	78.70	135.20	4.23	5.84	13.67	9.40	2.33	102.53	8.11
Phenotypic variance	101.90	29.08	42.25	28.46	0.11	0.68	6.25	0.18	0.08	1.98	0.50
Genotypic variance	84.88	24.95	36.09	11.08	0.05	0.50	2.77	0.14	0.01	0.32	0.17
PCV (%)	142.72	42.62	53.68	21.05	2.60	11.62	45.71	1.90	3.31	16.69	6.14
GCV (%)	118.88	36.57	45.86	8.20	1.08	8.53	20.27	1.52	0.52	2.71	2.06
Heritability (%)	83.29	85.82	85.43	38.93	41.41	73.46	44.34	80.37	15.60	16.23	33.56
Genetic advance	17.32	9.53	11.44	4.28	0.28	1.25	2.28	0.70	0.09	0.47	3.97
Genetic advance (%)	24.26	13.97	14.53	3.16	6.69	21.34	16.70	7.44	3.80	3.97	6.02

Note: PH = Plant height at maturity; IDF = Initial days of flowering; DF = Days to 50% flowering; DM = Days to maturity; PB/P = Primary branches/plant; SB/P = Secondary branches/plant; B/P = Bolls/plant; S/B = Seeds/boll; SY/P = Seed yield/plant; TSY = Total seed yield (g/m^2); TSW = Thousand seed weight; GCV = Genotypic coefficient of variation; Phenotypic coefficient of variation; Genetic advance (%) = Genetic advance as percent of the mean

Similarly, cluster III comprised of the shortest accessions (average 53.33 cm), early maturing and some of the low yielding collections. Cluster IV that contained the largest number of 24 accessions, was characterized by shorter plants, early maturing and good yielding collections. The remaining seven clusters (V-XI) that possessed one accession each, clearly displayed their considerable variability or distance relationship with others (Table 3.7) in the measured attributes and thus can be used to develop better performing cultivars in the future breeding programs.

Table 3.5. Eigenvectors and eigenvalues of the first 10 principal components for 11 different characters of 60 linseed accessions studied in the glasshouses at UFS (South Africa), 2000-2001

	Eigenvectors									
Character	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
PH	0.07	0.49	0.37	0.00	0.12	-0.12	-0.34	-0.07	0.68	-0.10
IDF	0.42	0.34	-0.06	-0.20	0.01	0.08	0.21	-0.37	-0.08	0.69
DF	0.47	0.25	-0.07	-0.19	-0.06	0.03	0.25	-0.27	-0.17	-0.71
DM	0.43	0.20	0.20	0.03	-0.01	-0.29	0.01	0.76	-0.26	0.08
PB/P	-0.08	0.33	-0.15	0.17	-0.80	0.25	-0.33	0.04	-0.13	0.01
SB/P	-0.11	0.31	-0.09	0.57	0.32	-0.35	-0.25	-0.29	-0.43	-0.02
B/P	-0.16	0.35	-0.19	0.45	0.15	0.39	0.57	0.25	0.22	-0.03
S/B	0.25	0.01	-0.55	-0.12	0.39	0.41	-0.51	0.19	0.03	-0.02
SY/P	-0.37	0.31	-0.20	-0.41	0.05	-0.19	0.06	0.10	-0.09	-0.02
TSW	-0.20	0.15	0.60	-0.16	0.22	0.57	-0.11	0.00	-0.41	-0.03
SY/PT	-0.38	0.31	-0.20	-0.40	0.05	-0.19	0.06	0.10	-0.08	-0.01
Eigenvalue	2.17	2.08	1.02	1.02	1.01	0.99	0.99	0.75	0.91	0.05
IVE (%)	19.75	18.95	9.30	9.28	9.15	9.03	8.98	6.81	8.28	0.47
CVE (%)	19.75	38.70	48.00	57.28	66.43	75.46	84.44	91.25	99.53	100.00

Note: PC = Principal components; IVE = Individual variability explained; CVE = Cumulative variability explained

Table 3.6. Cluster of 60 linseed accessions evaluated in glasshouses at UFS (South Africa), 2000-2001

Cluster	No. of collections	Name of collections and their original sources or collection areas
I	3	10037 (Sidamo), Omega (USA), N1266 (Canada)
II	23	10005 (Sidamo), 10008, 10169 and 10235 (Gonder), 10061 (Gamo-Gofa), 10068 and 10176 (Gojam), 10104, 10111, 10118, 10119, 10179, 10185, 10192 and 10197 (Shewa), Belay 96 (Holeta) 10138 (Welega), 10250, 10252, 10254, 10256, 10258 and 10260 (n.a.)
III	3	10080 (Harar), 10236 (Gonder), 10064 (Gamo-Gofa)
IV	24	10002 and 10109 (Shewa), 10010 and 10085 (Harar), 10026, 10072 and 10073 (Gojam) 10039 (Eritrea), 10041 and 10062 (Bale), 10046 (Welega), 10144 (Welo), 10120 (Sidamo), 10125 and 10042 (Tigray), 10159 and 10162 (Gonder), 10047 (Ilubabor) 10246 and 10248 (n.a.), Chilalo (Arsi), CI-1525 and CI-1652 (Europe), CDC-VG (Canada)
V	1	10007 (Kefa)
VI	1	10022 (Welo)
VII	1	10060 (Gonder)
VIII	1	10204 (Welo)
IX	1	10006 (Illubabor)
X	1	M20G (Canada)
XI	1	CDC1747 (Canada)

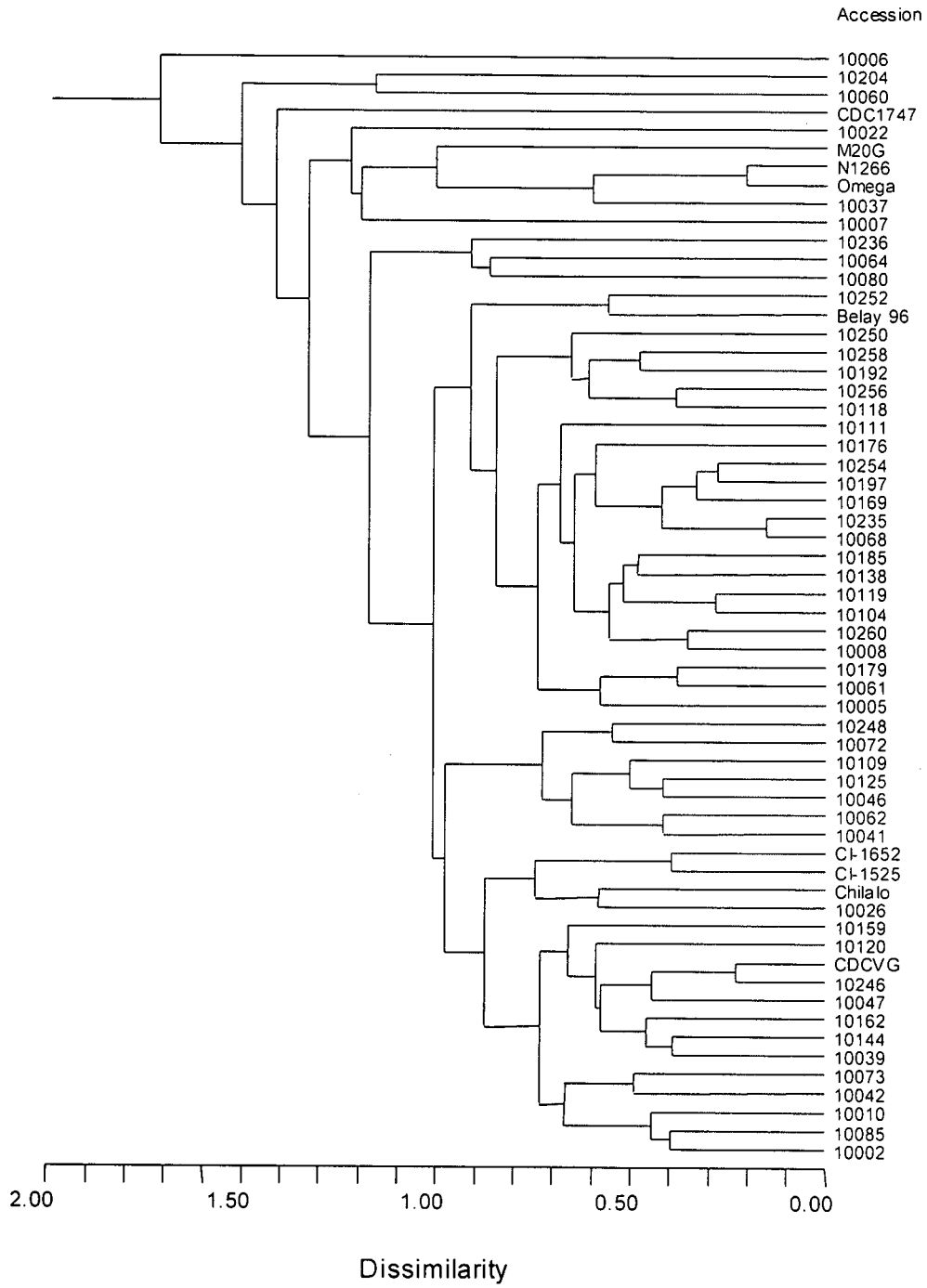


Fig. 3.2. Dendrogram showing clusters of 60 linseed accessions tested at UFS, 2000-2001

Table 3.7. Mean performance of each cluster analysis over the two years for 11 measured traits of linseed accessions studied in glasshouses at UFS (South Africa), 2000-2001

Cluster	PH	IDF	DF	DM	PB/P	SB/P	B/P	S/B	SY/P	TSY	TSW
I	83.33	67.00	75.33	133.67	5.00	7.00	17.33	9.00	2.26	114.00	6.15
II	69.39	71.13	82.45	136.91	4.22	5.65	13.26	9.91	2.33	107.13	7.90
III	53.33	61.00	70.67	124.33	4.33	5.33	12.00	9.33	2.49	77.20	8.00
IV	71.21	65.46	75.21	133.79	4.04	5.83	13.33	9.13	2.41	101.31	8.36
V	86.00	82.00	95.00	141.00	5.00	7.00	17.00	10.00	2.53	99.00	7.31
VI	94.00	71.00	85.00	148.00	5.00	5.00	11.00	9.00	2.36	87.00	8.05
VII	75.00	71.00	85.00	144.00	4.00	7.00	14.00	9.00	1.80	85.00	9.93
VIII	78.00	81.00	96.00	147.00	4.00	5.00	8.00	9.00	1.67	62.00	6.95
IX	57.00	58.00	66.00	123.00	5.00	7.00	18.00	9.00	2.96	109.50	7.57
X	92.00	73.00	80.00	137.00	5.00	7.00	19.00	9.00	2.90	137.50	9.84
XI	81.00	67.00	75.00	139.00	4.00	9.00	20.00	10.00	2.11	106.00	6.87

Note: PH = Plant height at maturity; IDF = Initial days of flowering; DF = Days to 50% flowering; DM = Days to maturity; PB/P = Primary branches/plant; SB/P = Secondary branches/plant; B/P = Bolls/plant; S/B = Seeds/bolls; SY/P = Seed yield/plant; TSY = Total seed yield (g/m^2); TSW = Thousand seed weight

3.5 Discussion

The analysis of variance showed highly significant differences (Tables 3.2 and 3.3) among the tested accessions for most of the studied traits, indicating the presence of substantial variability among these collections of Ethiopian linseed. Earlier characterizations also displayed a wide range of diversity among several collections from Ethiopia (Hiruy, 1987; Getinet *et al.*, 1987; Adefris *et al.*, 1992; Adugna, 2000). Appreciable ranges of variability were reported (Getinet *et al.*, 1987) for days to 50% flowering, days to 50% maturity, plant height, number of seeds/boll and 1000-seed weight. Most of the accessions were also noted to possess small boll size, light-blue petal colours and brown seed colours. Moreover, taller and late maturing linseed accessions were initially collected from higher altitudes of Ethiopia and they were susceptible to lodging (Getinet *et al.*, 1987). Some of the accessions were collected from sites ranging between 1680 m above sea level at Burie/ Ilubabor (western Ethiopia) and 3430 m at Werre-Himanu/ Welo (northern Ethiopia). Generally, the Ethiopian collections of linseed were observed with smaller seeds, shorter plant height, more prolific branching habits and lower oil contents than the exotic ones. However they possess tremendous variability for seed and oil yields, tolerance to biotic (rusts, wilt and pasmo diseases) and abiotic (frost and drought) stresses (Adefris *et al.*, 1992). Thus, the wide diversity detected among these linseed germplasm entails ample opportunities and prospects for the genetic improvement of the crop through either direct selection from the collections or following trait recombination via hybridisation of desirable parental lines.

The year-to-year variability and year x accession interaction was chiefly due to the temperature difference between the two glasshouses where the trials were undertaken. The glasshouse that was used in the first year had minimum and maximum temperatures of 13 and 35°C, respectively, while the one used during the second year was 8 to 25°C. Green (1986a) also demonstrated the significant genotype by temperature interaction for 1000-seed weight, oil content and fatty acid composition of linseed, indicating temperature-sensitive and stable genotypes in Australian cultivars. He also reported plants of the same genotype perform significantly different when exposed to different temperature treatments. Plants grown between 15/10°C and 18/13°C (night/day) were

reported (Green, 1986a) with normal growth and high yield, whereas those exposed to 30/25°C showed retarded growth and lower yields. Earlier studies (Yermanos and Goodin, 1965; Dybing and Zimmerman, 1965) also indicated drastic changes in the vegetative development and fatty acid composition of linseed as the result of changes in temperatures during pre-and post-flowering stages. Thus, the discrepancies in the extreme values of characters apparent in this study could be ascribed partially to the differences in the number and type of the tested accessions and partly to the year, and year x accession interaction effect (Table 3.3), as opposed to most of the previous single year tests (Getinet *et al.*, 1987; Adefris *et al.*, 1992).

As shown in Table 3.4, the phenotypic and genotypic variances and their respective coefficients of variability were high for plant height, days to flowering and maturity, and bolls/plant. Singh (1980) also found high genetic variability for plant height, boll/plant and branches/plant. Likewise, Mirza *et al.* (1996) and Khorgade and Pillai (1994) observed high phenotypic and genotypic coefficients of variation for boll/plant and plant height. In fact, plant height and primary branches/plant were reported (Jeswani *et al.*, 1970) to contribute maximum divergence and these traits were regarded as main characters to differentiate oilseed type of linseed from the fibre type (flax). Many other recent reports (Mahto and Mahto, 1998; Mishra and Yadav, 1999; Yadav and Gupta, 1999) also indicated high genetic coefficients of variation for bolls/plant, primary and secondary branches/plant, 1000-seed weight and seed yield/plant. However, these genetic coefficients of variation need to be studied together with estimates of heritability and genetic advance to acquire the best prediction of genetic advance expected from selection. To this effect, broad sense heritability was estimated to range between 15.60 and 86% and relatively higher percentages were recorded for days to initial flowering, days to 50% flowering, plant height, seeds/boll and secondary branches/plant (Table 3.4). Similar results were reported for seeds/boll, plant height, 1000-seed weight, days to flowering and maturity in India (Singh, 1984; Singh *et al.*, 1995; Mirza *et al.*, 1996; Mahto and Mahto, 1998).

Likewise, higher genetic advances were computed for plant height, secondary branches/plant, bolls/plant and days to flowering, indicating their expected good gains in the future selective breeding programs. Mahto and Mahto (1998) and Mishra and Yadav (1999) also found higher heritability values along with high genetic advance for days to flowering and maturity, plant height, and bolls/plant, indicating the predominance of additive gene action in the expression of these traits. Actually, plant breeding programs that are conducted under favourable environments are often considered to have higher selection gains owing to high heritabilities and large genotypic variance (Ceccarelli, 1996; vom Brocke *et al.*, 2002). In the current study, the additive component of genetic variance could not be separated from the variance among accessions that may have led to an overestimation of the genetic gain, especially for traits with a relatively high measure of dominance (vom Brocke *et al.*, 2002). Nevertheless, the results indicate that sufficient genetic variability exists in the tested collections for the progeny based selection. Moreover, high genetic variation provides the small-scale farmers with increased yield stability by reducing the chances of crop losses (Zeven, 2002) due to biotic and abiotic stresses. For the poor farmers, diversity is an insurance that can secure their survival against unpredictable crop failures.

The presence of wide diversity among the tested collections of linseed was also confirmed by the principal component analysis (Table 3.5), which indicated that the overall diversity could be explained by 10 eigenvectors. The first five eigenvectors were responsible for about 66.43% of the variability among the tested accessions. This analysis revealed that the major contributing characters are fairly distributed among days to flowering and maturity, plant height, bolls/plant, branches/plant and seed yield/plant, while plant height, bolls/plant and days flowering were the most useful in distinguishing the tested accessions. Kebebew *et al.* (1999) also reported that the principal component analysis grouped 20 variables of tef into 19 components that accounted for all variability. They further indicated that the first 10 eigenvectors explained about 90% of the entire variance, while the first five accounted for about 74%. The first eigenvector explained about 34% of the gross variability among the evaluated germplasm of tef, and this variability was due mainly to variation in plant height, primary tiller culm and panicle

length (Kebebew *et al.*, 1999). Cluster analysis further substantiated the existence of wide diversity among the 60 accessions, classifying them into 11 groups (Table 3.6 and Fig. 3.2) based on the analysis of 11 agro-morphological characters. The clustering pattern indicated that accessions in clusters V to XI were genetically more distant among themselves as well as from the other collections for they formed the most divergent single-genotyped clusters. Hence, they could be used in the crossing programs to develop more productive genotypes. No parallelism was observed between the geographical origins (collection areas) and genetic diversity of the studied accessions. For example, all clusters from I to IX consisted of different accessions collected from different parts of Ethiopia as well as some exotic ones in clusters IV and I. The wide ranges of various traits, such as days to maturity can offer great flexibility for the development of suitable varieties for the various agroecological zones of Ethiopia with different agro-climatic conditions. That is to say, different cultivars can be developed for specific agroecological regions to make use of the available potential of both the germplasm and the environment.

3.6 Conclusions

The current analyses of genetic diversity between accessions of linseed in Ethiopia indicate the presence of a wealth of genetic diversity for different characters that need be exploited with further exploration and investigation. The broad spectrum of variability observed among these collections of linseed for different characters implies better possibilities for genetic improvement of the crop through selection and cross breeding.

CHAPTER 4

GENETIC VARIABILITY AMONG LINSEED ACCESSIONS UNDER FIELD CONDITIONS IN ETHIOPIA

4.1 Abstract

A field experiment was conducted in a randomised complete block design with three replications at Holeta Research Centre, Ethiopia in 2000 and 2001. The main objectives were to assess the extent of genetic diversity among 60 accessions, to estimate phenotypic and genotypic variances, coefficients of variation, broad sense heritability and predicted genetic gains, and to cluster the accessions based on their similarity distance. The combined analyses of variances for the accessions were highly significant ($P < 0.01$) nearly for all characters. The phenotypic coefficient of variation varied from 6.26% for days to maturity to 54.97% for yield/plant, whereas that of genotypic ranged between 5.46% and 50.18% for the same characters. Estimates of heritability differed from 67.38 for seeds/boll to 91.38% for days to 50% flowering. Values of predicted genetic advance (as percent of mean) varied from 9.82% for days to maturity to 94.37% for yield/plant. Among the 12 principal components involved in explaining the entire variation, the first 10 had eigenvalues of 93.74% of the total variance. The third principal component, which accounted for about 14% of the entire variability, was due mainly to bolls/pant and branches/plant. Cluster analysis grouped the 60 accessions into nine major classes consisting of one to 39 collections, indicating that efficient management of germplasm is possible by eliminating redundant accessions. The study also demonstrates the existence of high diversity among the evaluated germplasm that could be utilised in future genetic improvement of this crop. Further collection and investigation are needed, targeting specific areas, such as northern Ethiopia.

4.2 Introduction

Genetic diversity can be measured as the number of different factors and their relative frequency at genus, species, population, individual, genome, locus and DNA base sequence levels (Kresovich and McFerson, 1992; Gaston, 1998; Kumar, 1999). However, the process of measurement needs to be iterative and dynamic because micro- and macro evolutionary changes will occur everywhere (Gaston, 1998). Cost-effective detection of variation and employment of the appropriate tool, are also the key factors in the assessment processes for genetic representation and accession uniqueness based on genetic distance. This becomes even more important nowadays and in the future as concern for property rights of plant genetic resources increases locally and globally (Kresovich and McFerson, 1992; Blakeney, 2002). Since genetically diverse parents are likely to produce high heterotic effects and yield desirable segregates, genetic divergence plays a vital role in the building of a successful breeding program. Thus, quantitative assessment of genetic divergence is necessary to determine the nature and extent of genetic differences among germplasm of vital crops including linseed thereby to maintain and exploit their genetic resources efficiently.

Linseed (*Linum usitatissimum* L.) is an annual field crop that is largely grown in temperate climates (Mansby *et al.*, 2000) and cool tropics including the highlands (>2500 meters above sea level) of Ethiopia. It is the second most important oil crop in the highlands of Ethiopia in terms of area and production (Adefris *et al.*, 1992; Adugna and Labuschagne, 2002). The main linseed growing areas of Ethiopia are the south-eastern regions of Arisi, Bale, eastern Welega, eastern Gojam, Semen mountains, Tigray, western Welo and highlands of Hararghe and Shewa (Adugna, 2000). The principal regions of linseed production have an altitude range of 1200 to 3500 meters above sea level and the crop performs best within 2200-2800 m. Linseed requires cool temperatures during its growing period to produce good yields. The mean temperature can range from 10°C to 30°C although the crop grows best within 21-22°C. The crop also grows very well within a 12 to 18 hour photoperiod (Adefris *et al.*, 1992). In deed, linseed has been cultivated in Ethiopia since antiquity (Seegler, 1983; Adefris *et al.*, 1992). Nevertheless,

its productivity has been very low (less than 500 kg/ha) due mainly to poor cultivars that are susceptible to weeds, diseases and insect pests, besides inadequate cultural practices.

Linseed has been cultivated in Ethiopia for two primary purposes, seed and oil production. It has traditionally been used for food and as a cash crop since ancient times (Seegler, 1983). Linseed oil has many food and industrial applications (Rowland, 1990), and its seed cake is also a valuable feed for livestock (Pizzey, 1998). Linseed oil has 55-58% of unique alfa-linolenic fatty acid (an essential omega 3 fatty acid), which has beneficial effects on health and the auto immune system (Carter, 1993; Aldrich, 1998). Alpha-linolenic was reported (Carter, 1993; Flax Council of Canada, 2000b; Payne, 2000) to anti-hypercholestrolemic and anti-carcinogenic effects and is important for the normal development of brain and retinal tissues of babies. The soluble fibre of linseed helps to lower blood cholesterol, whereas insoluble fibre promotes laxative effects (Payne, 2000). Moreover, its lignan (phytoestrogen, plant compound with estrogen-like activity) were found useful for health of women (Flax Council of Canada, 2000b; Payne, 2000). Consequently, linseed is currently becoming popular worldwide for its functional food products.

Collection and characterization of linseed has been underway in Ethiopia at Holetta Research Centre since the early 1980s (Getinet *et al.*, 1987) in collaboration with the Institute of Biodiversity Conservation and Research, IBCR (then Plant Genetic Resource Centre of Ethiopia). As a result, some 641 collections were maintained at the IBCR (Abebe *et al.*, 1992). The characterization activities were done to select and advance elite materials, and also to rejuvenate the germplasm collections. Nonetheless, the characterization was conducted only at one location for one season without in depth analysis. Moreover, only simple descriptive statistics (range, mean, standard deviation and frequency distribution of major traits) were employed to analyse their data. Systematic and detailed studies were not done to generate adequate information that is required by the current and future breeding programs. Although the country is considered among a centre of diversity for linseed (Seegler, 1983; Adugna and Adefris 1995), and the wide range of agro-climatic conditions in the country (MoA, 1998;

Adugna, 2000) may have continued to its further diversity. But in depth studies were not done to make use of important genes for the current and future breeding programs. In other words, information on the extent of genetic diversity of linseed in Ethiopia is very meagre and this study was undertaken to address such problems. Thus, the specific objectives of this study were: a) to assess the extent of diversity among 60 accessions of linseed for agro-morphologically useful traits under field conditions in Ethiopia; b) to quantify the phenotypic and genotypic variances and coefficients of variation among the accessions; c) to estimate heritability (broad sense) and predicted genetic advances of the traits, and d) to identify and cluster the accessions based on their genetic distances for effective utilization in the breeding programs. The basic assumption is that by analysing and broadening the germplam basis, more productive genotypes could be developed in a sustainable manner, and this in turn could contribute positively to the strengthening of future improvement and conservation efforts.

4.3 Materials and Methods

Plant materials

The accessions used in this study were selectively taken from the Highland Oil Crops Research Program of Holeta Research Centre in the Ethiopian Agricultural Research Organization to represent the apparent diversity of linseed germplasm in the country. The accessions were collected by the IBCR in collaboration with the Highland Oil Crops Research Program since the early 1970s. The germplasm collections are preserved at the IBCR and some of their duplicates are also kept at Holeta for research purposes. Totally 60 accessions, including four released varieties (Chilalo, CI-1525, CI-1652 and Belay 96) were studied for their variability and genetic divergence. Belay 96 was developed out of a crossing carried out between locally well-adapted and disease resistant collections at Holeta Research Centre and it was released in 1996. Fifty-three accessions were collected from different areas of Ethiopia and Eritrea (Figure 3.1), the remaining seven being introductions from Europe, USA and Canada (Table 3.1).

Experimental site and methods

The study was conducted at Holeta Research Centre (latitude 9° 3' N, longitude 38° 30' E, altitude 2400 m above sea level) during the years 2000 and 2001 main cropping seasons. The 60 accessions were evaluated in two rows (5 m long spaced at 20 cm) that were laid out in a randomised complete block design with three replications. The seeds were planted at a seeding depth of about 2.5 cm on June 26 during both years. The recommended fertilizers of both nitrogen (N) in the form of urea and phosphorus (P_2O_5) in the form of diammonium phosphate (DAP) were manually drilled and incorporated with the soil both at a rate of 23 kg/ha at planting. The seeds were also drilled by hand at a rate of 25 kg/ha. Weeds were controlled by hand weeding about 2-3 times as required. Neither herbicides nor insecticides were applied, as there was no need. The plants of each accession was separately harvested and dried for about 15 to 30 days, threshed and cleaned manually. Seed yield data were taken at about 8% seed moisture level.

Data on the agronomical and morphological characters were collected from five randomly selected plants and their means were recorded for all observations. Except for 50% flowering, disease scores and similar observations, most of the data were recorded at maturity and thereafter. Scores for powdery mildew and pasmo diseases were recorded on 0-5 scales (0 = nil; 5 = very severe) under normal field conditions without any artificial inoculation. Days to flowering and maturity represent the number of days from sowing to 50% blooming and ripening, respectively. The unit of measurement for plant height was centimetres (cm), while that of seed yield and 1000-seed weight was in grams (g). In general, most of the evaluation was undertaken based on the major phenological and morphological quantitative descriptors (Diederichsen, 2001) that are reported to display high heritability values.

Statistical analysis

The mean values of all observations for the 12 characters (interval and ordinal variables) were first screened for normality and outliers by using the Number Cruncher Statistical System, NCSS 2000 (Hintze, 1998) statistical system for Windows. Then

data that showed non-normal distribution were transformed by a square root system and analysed for their variance and significance using AGROBASE 98 software (Agronomix Software, Inc., 1998). The critical difference (CD) was also calculated for comparing means of the accessions. Average data over the two years were used in defining some basic statistics, such as range and mean. The same mean data were also used for the multivariate analyses of principal components and clustering by which classification of the accessions was investigated. In employing the analyses of both principal components and clustering, the 60 accessions were considered as operational taxonomic units and they were represented by rows and the measured characters were taken as variables by columns. Both the principal component and cluster analyses were performed by NCSS 2000 (Hintze, 1998). In the process of hierarchical clustering, unweighted pair group method of arithmetic average (UPGMA), Manhatan distance and the standard deviation scaling type were used.

Estimation of genetic parameters were done to identify and ascertain the genetic variability among accessions and to determine the extents of environmental effects on various characters. The variance components were based on the combined analyses over the two years and they were used in partitioning the gross (phenotypic) variability into its parts due to genotypic and environmental factors, as indicated by Kebebew *et al.* (1999). Moreover, these genetic parameters were estimated by adapting the formulae suggested by Allard (1960), Singh and Chaudhary (1977), Kebebew *et al.* (1999) and Atlin *et al.* (2001). Accordingly, genotypic and phenotypic components of variance and their coefficients of variability were estimated by applying the following formulae: (i) Genotypic variance, GV = MSa - (MSay/y - MSe/ry), where MSa = Mean square of accessions, MSay = Mean square of accession by year interaction; MSe = Mean square of error, r = number of replications; y = number of years (ii) Phenotypic variance, PV = GV + (MSay/y + MSe/ry); (iii) Genotypic coefficient of variability, GCV% = (GV/grand mean) x 100; (iv) Phenotypic coefficient of variability, PCV% = (PV/grand mean) x 100; (v) Heritability (broad sense) for all the characters were computed as H = GV/PV x 100; (vi) Genetic advance, GA = H x PS x

K, where PS = phenotypic standard deviation ($\sqrt{PV}$), and K is selection differential at 5 percent selection intensity = 2.06; GA% (as % of mean) = GA/grand mean x 100.

4.4 Results

The combined analysis of variance across the two years showed that there were significant ($P < 0.01$) variations among the entries or accessions for almost all the characters (Table 4.1). These apparent differences may suggest the presence of adequate genetic diversity between the accessions. The difference across the two years was also highly significant due mainly to variations in soil and climatic conditions. However, year x accession interaction was largely non-significant except for seed yield and plant height. The significant interaction indicates the differential responses of the accessions over the growing environments. The environment often influences economic characters, such as yield that is mostly controlled by many genes and has a complex type of inheritance (Mishra and Yadav, 1999). The wide variability between the accessions was also revealed by their mean performance (Table 4.2). Plant height ranged from 58-86 cm, while days to 50% flowering and maturity were 74-96 and 146-162 days, respectively. Similarly, a broad spectrum of variability was noted for the number of bolls/plant, seed yield/plant and total yield (g/m^2). The highest yield was obtained from CDC-VG, followed by that of Belay 96, CI-1652 and Chilalo. In a nutshell, the large differences between the minimum and maximum values of all 12 characters and the statistically significant difference imply the presence of substantial variability among the studied accessions.

The estimated phenotypic variance ranged from 0.17 for seed yield per plant to 4500 for the total seed yield (Table 4.3). Similarly, the genotypic variance ranged from 0.14 to 3206.83 for the same two characters, respectively. The next highest genetic variances were recoded for plant height (282.32), days to 50% flowering (181.78) and days to maturity (69.00). These variances again demonstrate the existence of considerable variability in the studied characters. Furthermore, the phenotypic coefficient of variation varied from 6.26% for days to maturity to 54.97% for seed yield/plant. Genotypic coefficient of variation (GCV) was also lowest (5.46%) for

days to maturity and highest (50.18%) for yield/plant. The other traits, which had relatively larger GCV percentages, were seed yield (g/m^2), bolls/plant, secondary branches/plant and plant height. These larger percentages of both phenotypic and genotypic values were due to their respective larger variances and lower means, as shown in Table 4.3. In other words, traits that had relatively larger genetic variances also showed higher genotypic coefficients of variation, suggesting that selection for these characters might be more effective than the remaining ones since they had less environmental influences.

Table 4.1. Mean squares of the combined analysis of variance across two years for the measured traits of 60 linseed accessions evaluated under field condition at Holeta Research Centre (HRC) in Ethiopia, 2000-2001

		Characters											
Source	Df	PH	PM	PS	DF	DM	PB/P	SB/P	B/P	S/B	SY/P	TSY	TSW
Year (Y)	1	9891.03**	13.03**	5.09**	98.18*	4410.00**	5.63**	16.04*	238.47**	14.80*	1.12**	404278**	0.18
R(Y)	4	1333.02	15.33	3.08	39.04	785.02	4.73	13.82	171.31	8.22	0.39	54526	0.11
Entry (E)	59	319.67**	1.02**	0.81**	198.93**	90.61**	1.09**	5.04**	60.76**	3.29	0.17**	4500**	4.88**
Y x E	59	61.26*	0.16	0.29	26.23	33.89	0.44	1.32	18.75	1.22	0.39	13.74**	1.49
Residual	236	40.31	0.31	0.32	24.23	27.97	0.55	2.46	27.14	2.78	0.05	1165	2.11
Range		58-86	1-3	1-2	74-97	146-162	3-6	4-9	15-31	6-10	0.48-1.22	75-211	7-10
R^2		79.85	66.06	51.93	70.30	69.29	46.77	43.53	46.72	32.36	53.81	78.73	43.03
CV (%)		9.21	22.07	24.44	5.75	3.48	17.46	18.15	20.39	18.90	20.64	21.37	17.50

*, ** = Significant at $P < 0.05$, 0.01, respectively; df = degree of freedom; PH = Plant height at maturity; PM = Powdery mildew score; PS = Pasmo score; DF = Days to 50% flowering; DM = Days to maturity; PB/P = Primary branches/plant; SB/P = Secondary branches/plant; B/P = bolls/plant; S/B = Seeds/boll; SY/P = Seed yield/plant; TSY = Total Seed Yield (g/m^2); TSW = Thousand seed weight; R = Replications; R^2 = Repeatability; CV = Coefficient of variation.

Table 4.2. Mean performance of 12 characters for 60 linseed accessions evaluated under field conditions at HRC (Ethiopia), 2000-2001

	Characters											
Accession	DF	DM	PM	PS	HT	PB/P	SB/P	B/P	SY/P	TSY	S/B	TSW
1. 10002	89	151	2	1	62	4	6	16	0.55	70.00	10	3.73
2. 10005	89	150	2	2	60	4	6	20	0.52	75.50	9	3.66
3. 10007	90	158	2	1	77	5	8	24	0.70	78.00	9	3.56
4. 10008	91	151	2	1	70	4	8	26	0.83	80.50	10	3.71
5. 10010	79	151	1	2	65	5	8	26	0.80	63.00	9	3.83
6. 10022	96	158	2	1	72	4	6	16	0.60	77.00	9	4.17
7. 10026	88	149	1	1	64	4	6	20	0.55	69.50	9	3.63
8. 10037	78	150	1	2	73	4	6	20	0.82	72.00	9	4.14
9. 10039	78	146	2	2	67	4	7	22	0.80	79.50	8	4.49
10.Chilalo	85	154	2	2	82	3	5	18	0.62	92.50	9	4.80
11. 10041	82	151	2	2	70	4	7	26	0.73	55.00	9	4.17
12. 10046	77	148	2	2	67	4	7	23	0.67	68.00	9	4.19
13. 10060	95	159	2	1	72	4	7	16	0.57	59.00	9	4.16
14. 10061	88	150	2	1	63	4	7	19	0.48	71.50	9	3.28
15. 10068	90	149	2	1	69	6	9	24	0.67	88.50	8	3.94
16. 10080	80	147	2	2	63	4	7	22	0.65	37.50	8	3.81
17. 10085	89	155	1	2	67	5	6	19	0.58	59.50	7	4.80
18. 10104	90	155	2	1	71	4	8	24	0.58	78.50	9	3.88
19. 10109	77	147	2	2	61	4	6	19	0.48	62.50	9	4.39
20.CI-1525	87	162	1	2	85	4	7	26	1.05	84.50	8	4.94
21. 10111	90	153	2	2	63	4	7	19	0.52	77.50	9	3.84
22. 10118	89	152	2	2	71	5	7	24	0.57	66.00	8	3.69
23. 10119	90	151	2	2	69	4	7	18	0.57	68.00	9	3.53
24. 10120	86	149	2	2	72	4	6	18	0.65	73.50	9	4.44
25. 10125	90	147	2	2	61	4	6	21	0.77	52.00	8	4.02
26. 10138	89	151	2	2	61	4	7	20	0.55	71.00	8	3.72
27. 10144	82	153	2	2	62	5	8	18	0.55	60.00	9	4.47
28. 10159	77	162	2	1	73	4	6	16	0.62	62.50	9	4.29
29. 10162	74	151	2	2	70	4	8	23	0.77	56.50	10	4.42
30.CI-1652	83	162	1	2	82	4	7	24	0.87	95.50	9	4.54
31. 10169	91	150	1	1	70	4	6	18	0.55	53.00	10	3.77
32. 10176	92	152	2	1	68	5	7	20	0.52	80.00	8	4.14
33. 10179	89	152	2	1	63	5	7	19	0.65	59.50	9	4.60
34. 10185	87	149	2	1	69	4	7	21	0.70	57.00	10	4.48
35. 10192	93	151	2	2	64	5	8	20	0.52	65.00	10	3.67
36. 10197	92	152	3	1	64	5	7	17	0.50	73.50	9	3.99
37. 10204	79	155	3	1	62	3	7	18	0.57	39.00	8	3.92
38. 10006	79	151	2	1	66	4	8	30	0.85	54.50	7	3.67
39. 10042	79	149	2	2	64	4	6	22	0.82	76.00	9	4.15
40. Belay 96	83	152	1	2	79	4	5	21	0.80	96.00	9	4.55
41. 10047	78	153	2	1	77	4	8	21	0.63	45.50	9	3.88
42. 10062	79	151	1	2	80	4	6	21	0.87	78.50	9	5.14
43. 10235	91	153	2	2	70	5	6	24	0.62	69.00	10	4.24
44. 10236	76	148	2	2	63	4	5	21	0.62	53.50	7	4.40
45. 10064	80	149	2	2	60	5	8	25	0.70	56.50	10	3.39
46. 10072	86	151	2	2	62	4	6	20	0.52	80.00	9	3.79
47. 10073	86	150	2	2	72	5	5	20	0.68	79.50	9	4.46
48. 10246	76	146	2	2	58	5	6	23	0.70	66.50	7	4.15
49. 10248	87	149	2	2	63	5	6	21	0.90	73.50	9	3.85
50. 10250	94	156	2	1	62	5	6	21	0.90	49.50	9	4.08
51. 10252	91	154	2	2	68	5	6	20	0.63	76.50	9	4.30
52. 10254	86	150	2	2	62	4	5	20	0.53	63.50	9	4.13
53. 10256	78	150	2	2	64	4	5	23	0.65	68.50	10	4.00
54. 10258	86	152	2	2	63	4	6	22	0.67	79.00	9	3.80
55. 10260	94	150	2	2	68	4	6	25	1.02	52.50	10	3.83
56.Omega	88	155	2	1	84	5	5	27	1.13	85.50	9	3.82
57. N1266	81	157	2	1	86	5	6	31	1.22	71.50	9	5.16
58. M20G	89	158	1	1	80	4	6	24	0.92	84.50	8	4.94
59.CDC1747	89	157	1	1	80	5	7	22	1.02	88.00	9	4.96
60. CDCVG	91	158	1	1	82	5	6	20	0.83	105.50	10	4.93
Mean	85.63	152.20	1.83	1.60	68.95	4.33	6.57	21.40	0.70	69.75	8.88	4.14
CD 0.05	6.66	7.16	0.75	0.54	8.60	1.00	2.12	7.05	0.30	23.10	2.26	1.97

The predicted broad sense heritability values of the 10 characters varied from 67.38 to 91.38%, the lowest value being recorded for seeds/boll and the highest for days to 50% flowering. Traits such as plant height, seed yield/plant, secondary branches/plant, 1000-seed weight and bolls/plant also showed higher heritability values (77-88%) than other characters (Table 4.3). The result suggests that selecting for traits with high heritability values could lead to better progress than those with lower heritability, as the latter were more influenced by environment than the former. On the other hand, characters with relatively lower heritability, such as seeds/boll, total seed yield and primary branches/plant may have poor response to selection due to substantial effects of the environment. Thus, broad sense heritability would be more reliable if accompanied by a high predicted genetic advance (Geleta, 1998; Kebebew *et al.*, 1999), and if its values have shown considerable stability across environments (Patil *et al.*, 1981).

Genetic advances (as a percent of mean) that might be expected from selecting the top 5% of the genotypes, ranged from 9.82% for days to maturity to 94.37% for yield/plant (Table 4.3). Similarly, higher percentages (47-70%) of genetic advances were predicted for total yield, bolls/plant, secondary branches/plant and plant height. Larger values of genotypic coefficient of variation together with high heritability and high genetic advance were reported (Patil *et al.*, 1981; Geleta, 1998; Kebebew *et al.*, 1999) to give better information than a single parameter alone. Hence, traits, like yield/plant, number of bolls/plant, days to 50% flowering and plant height that showed a high genotypic coefficient of variation, heritability and genetic advance would be very useful as a base for selection. Though seed yield (g/m^2) revealed a high genotypic coefficient of variation and genetic advance, its heritability was low due to significant influence of environment.

Table 4.3. Estimates of genetic parameters for 10 traits of 60 linseed accessions evaluated under field conditions at HRC (Ethiopia), 2000-2001

	Genetic parameters										
Trait	Mean	MSa	MSay	MSe	GV	PV	GCV%	PCV%	H%	GA	GA%
Plant height (cm)	68.95	319.67	61.26	40.31	282.32	319.67	24.53	26.11	88.32	32.53	47.49
Days to flowering	85.63	198.93	26.23	24.23	181.78	198.93	15.76	16.48	91.38	26.55	31.03
Days to maturity	152.20	90.61	33.89	27.97	69.00	90.61	5.46	6.26	76.15	14.93	9.82
Primary braches/plant	4.33	1.09	0.44	0.55	0.78	1.09	20.71	24.51	71.41	1.54	36.05
Secondary branches/p	6.57	5.04	1.32	2.46	3.97	5.04	30.70	34.59	78.78	3.64	56.13
Bolls/plant	21.40	60.76	18.75	27.14	46.86	60.76	32.05	36.49	77.13	12.38	57.98
Seeds/boll	8.88	3.29	1.22	2.78	2.22	3.29	16.90	20.59	67.38	2.52	28.58
Seed yield/plant (g)	0.70	0.17	0.04	0.05	0.14	0.17	50.18	54.97	83.33	0.71	94.37
Total seed yield (g/m^2)	69.75	4500	2198.04	1164.98	3206.83	4500.00	40.43	47.89	71.26	98.48	70.30
1000-seed weight (g)	4.14	4.88	1.49	2.11	3.78	4.88	23.46	26.65	77.53	3.53	42.56

Note: Msa = Mean square of accessions; MSay = Mean square of accession by year interaction; Mse = Mean square of error; GV = Genotypic variance; PV = Phenotypic variance; GCV = Genotypic coefficient of variation; PCV = Phenotypic coefficient of variation; H = Heritability; GA = Genetic advance; GA% = Genetic advance (as percent of mean)

Table 4.4. Eigenvectors and eigenvalues of the first 12 principal components for different characters of 60 linseed accessions tested at HRC (Ethiopia), 2000-2001

	Eigenvectors											
Characters	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
DF	-0.06	-0.57	-0.09	0.27	0.11	0.22	0.31	-0.19	-0.37	0.50	-0.08	0.07
DM	-0.36	-0.31	-0.08	-0.36	0.09	-0.09	-0.02	0.13	-0.52	-0.49	0.26	0.16
PM	0.34	-0.13	0.13	-0.20	-0.03	0.53	-0.14	0.70	-0.09	0.09	-0.03	-0.07
PS	0.11	0.48	-0.15	0.43	-0.04	-0.20	0.02	0.25	-0.64	0.03	-0.06	-0.14
HT	-0.50	-0.04	-0.04	-0.17	-0.12	-0.05	0.02	0.16	0.01	0.03	-0.74	-0.37
PB/P	-0.10	-0.21	0.38	0.54	0.38	0.13	-0.47	-0.04	0.03	-0.32	-0.13	-0.10
SB/P	0.09	-0.22	0.53	-0.15	-0.01	-0.65	-0.11	0.18	-0.07	0.36	0.13	-0.14
B/P	-0.21	0.24	0.58	0.07	-0.19	0.14	0.29	0.05	-0.07	-0.02	-0.18	0.61
SY/P	-0.41	0.21	0.29	-0.01	-0.19	0.36	0.03	-0.15	-0.07	0.15	0.48	-0.51
TSY	-0.35	-0.12	-0.19	0.39	0.05	-0.16	0.33	0.55	0.40	-0.02	0.27	0.04
S/B	-0.03	-0.26	-0.12	0.24	-0.82	-0.04	-0.40	-0.01	0.00	-0.04	0.04	0.13
TSW	-0.38	0.22	-0.22	-0.11	0.25	0.04	-0.55	0.08	0.00	0.49	0.08	0.36
Eigenvalue	1.15	1.04	1.65	1.01	1.02	1.04	1.09	1.08	0.99	1.18	0.47	0.28
IVE (%)	9.61	8.66	13.72	8.43	8.48	8.66	9.10	8.98	8.24	9.85	3.90	2.36
CVE (%)	9.61	18.26	31.99	40.42	48.91	57.57	66.67	75.65	83.89	93.74	97.64	100.00

Note: PC = Principal components; DF = Days to 50% flowering; PM = Powdery mildew score; PS = Pasmo score; PH = Plant height at maturity; DM = Days to maturity; PB/P = Primary branches/plant; SB/P = Secondary branches/plant; B/P = Bolls/plant; S/B = Seeds/boll; SY/P = Seed yield/plant; TSY = Total Seed Yield (g/m^2); TSW = Thousand seed weight; IVE = Individual variation explained; CVE = Cumulative variation explained

The principal component analysis grouped the studied variables into 12 components that accounted for the entire variability of the accessions. It also revealed that the first 10 eigenvectors explained 93.74% of the total variance. Of these, the first eight (the ones with eigenvalues greater than one) accounted for a cumulative amount of about 75.65%, while the first five accounted for about 49% of the total variability among the accessions (Table 4.4). The third principal component, which individually explained about 14% of the entire variability among the collections, has been due mainly to differences in the number of bolls/plant and secondary branches/plant. The first principal component that explained about 10% of the total variability of the accessions originated primarily from variations in scores of powdery mildew and pasmo diseases (Table 4.4). Likewise, the 10^{th} principal component, which accounted for 9.85% of the variation among the accessions, resulted largely from variability in days to 50% flowering, 1000-seed weight and secondary branches/plant. The remaining principal components, from the 2^{nd} to 8^{th} each accounted about 8% of the variation among the accessions arising from different plant characters.

The analysis of clustering has grouped the 60 accessions into nine categories (Table 4.5 and Fig. 4.1). Cluster I consisted of three accessions that were collected from northern and northwestern Ethiopia, while cluster II had two introductions from USA and Canada. Similarly, cluster III and V had four accessions each, collected from different regions of Ethiopia, whereas cluster IV was composed of those from exotic origins. Cluster VI consisted of the highest number of 39 accessions all collected from different areas of Ethiopia except one from Eritrea. The remaining three clusters had one accession each, indicating their distant relationships from the others. Though some of the exotic genotypes were grouped into clusters II and IV, the majority of the local collections were not necessarily clustered according to their geographic origins or specific collection sites. For example, accessions collected from Gonder was grouped in clusters I, III and VI. This shows that the clustering phenomenon was not necessarily based on the geographic origins of the collections.

Accessions in cluster I were generally characterized by intermediate plant heights, early maturing, tolerance to powdery mildew and pasmo diseases (Table 4.6). Cluster II that consisted of two accessions, had a common feature of the tallest plants, late maturing, highest number of bolls/plant and the top yielder per plant (1.18 g). Similarly, cluster IV comprised of tall, late maturing, high yielding, resistance to powdery mildew and pasmo diseases besides their heaviest 1000-seeds (4.86 g). All of the genotypes in this cluster were of exotic origin. On the other hand, cluster VI that possessed the largest number of 39 accessions, was characterized by shorter plants, early maturing and fairly good number of bolls/plant. The remaining three clusters (VII-IX) that contained one accession each, clearly displayed their distant relationship with others (Table 4.6), consisting of short, early maturing, and low yielding collections.

Table 4.5. Clusters of 60 linseed accessions evaluated at HRC (Ethiopia), 2000-2001

Cluster	No. of access.	Name of accessions and their original sources or collection areas
I	3	10022 (Welo), 10060 and 10159 (Gonder)
II	2	Omega (USA), N1266 (Canada)
III	4	10007 (Kefa), 10008 (Gonder), 10068 (Gojam), 10104 (Shewa)
IV	5	CI-1525 and CI-1652 (Europe), M20G, CDC-1747 and CDC-VG (Canada)
V	4	10037 (Sidamo), Chilalo (Arsi), Belay 96 (Holeta), 10062 (Bale)
VI	39	10002, 10109, 10111, 10118, 10119, 10179, 10185, 10192 and 10197 (Shewa), 10010 and 10080 (Hararge), 10005 and 10120 (Sidamo), 10026, 10072, 10073 and 10176 (Gojam), 10039 (Eritrea), 10041 (Bale), 10046 and 10138 (Welega), 10144 (Welo), 10061 and 10064 (Gamo-Gofa), 10162, 10169, 10235 and 10236 (Gonder), 10246, 10248, 10250, 10252, 10254, 10256, 10258 and 10260 (Eth./n.a.*), 10125 and 10042 (Tigray), 10047 (Ilubabor).
VII	1	10085 (Hararge)
VIII	1	10204 (Welo)
IX	1	10006 (Ilubabor)

* n.a. = not available

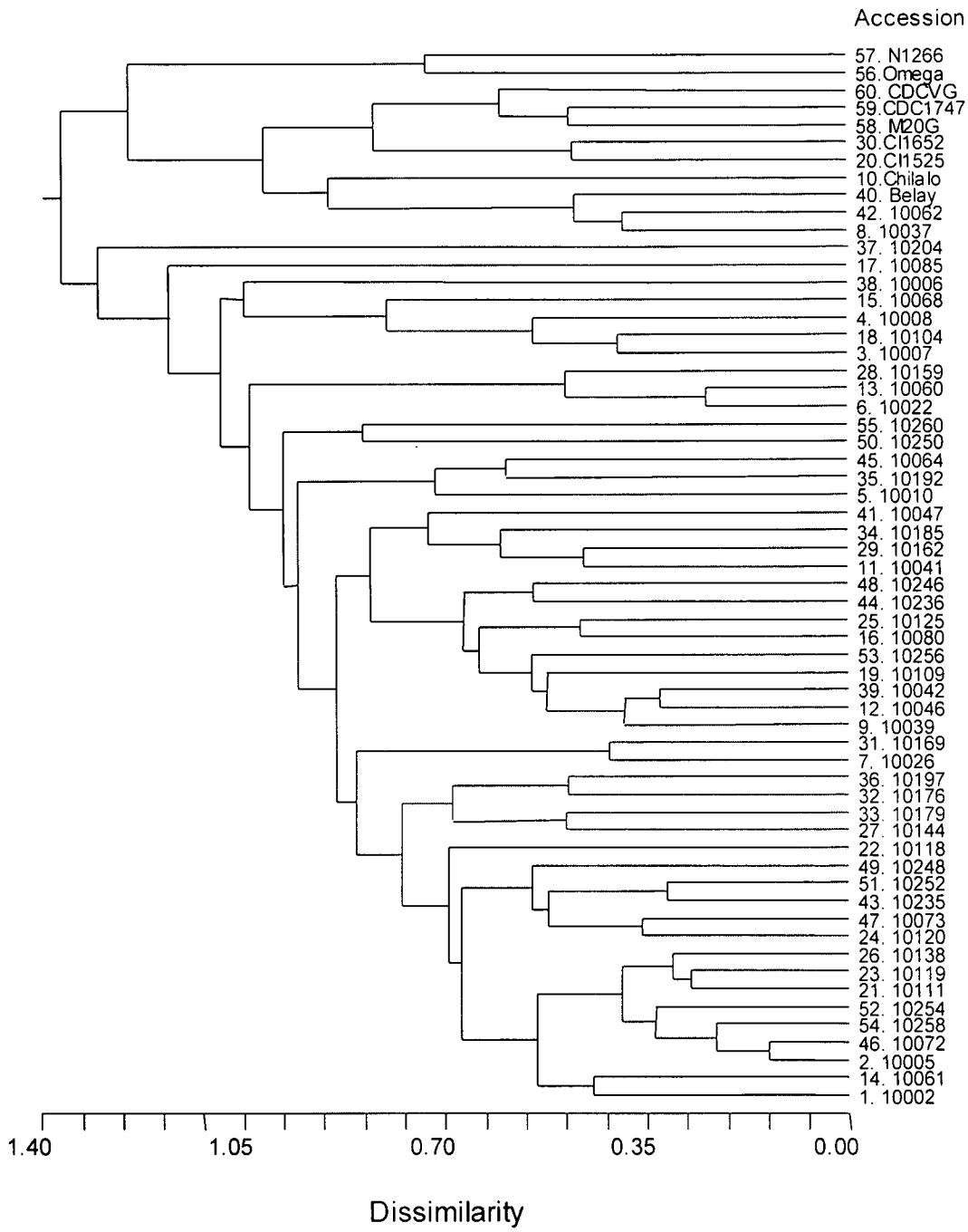


Fig. 4.1. Dendrogram illustrating clusters of 60 linseed accessions tested at HRC, 2000-2001

Table 4.6. Mean performance of nine clusters of 60 linseed accessions evaluated under field conditions at HRC (Ethiopia), 2000-2001

Characters	Clusters								
	I	II	III	IV	V	VI	VII	VIII	IX
Plant height (cm)	68.95	85.00	71.75	81.80	78.50	65.26	67.00	62.00	66.00
Powder mildew score	1.83	2.00	2.00	1.00	1.25	1.95	1.00	3.00	2.00
Pasmo score	1.60	1.00	1.00	1.40	2.00	1.74	2.00	1.00	1.00
Days to flowering	85.63	84.50	90.25	87.80	81.25	85.36	89.00	79.00	79.00
Days to maturity	152.20	156.00	153.25	159.40	151.75	150.33	155.00	155.00	151.00
Prim. braches/plant	4.33	5.00	4.75	4.40	3.75	4.36	5.00	3.00	4.00
Sec. branches/plant	6.57	5.50	8.25	6.60	5.50	6.54	6.00	7.00	8.00
Bolls/plant	21.40	29.00	24.50	23.20	20.00	20.95	19.00	18.00	30.00
Seeds/boll	8.88	9.00	9.00	8.80	9.00	8.97	7.00	8.00	7.00
Seed yield/plant (g)	0.70	1.18	0.70	0.94	0.78	0.65	0.58	0.57	0.85
Seed yield (g/m^2)	69.75	78.50	81.38	91.60	84.75	65.49	59.50	39.00	54.50
1000-seed weight (g)	4.12	4.49	3.77	4.86	4.66	4.01	4.80	3.92	3.67

4.5 Discussion

The combined analysis of variance across the two growing seasons showed highly significant ($P < 0.01$) difference between the tested accessions for most of the studied traits (Table 4.1). There was also a wide range of difference between the maximum and minimum values of the morphometric characters (Tables 4.2). This considerable variability could be ascribed partly to the differences in the evaluated accessions and partly to the year and year x accession interaction effects. Previous characterizations of linseed also displayed a wide range of variability among several collections in Ethiopia (Hiruy, 1987; Getinet *et al.*, 1987; Adefris *et al.*, 1992; Adugna, 2000). Broad ranges of variability were reported (Getinet *et al.*, 1987) for days to 50% flowering, days to 50% maturity, plant height, number of seeds/boll and 1000-seed weight. Most of the Ethiopian accessions were also observed (Adefris *et al.*, 1992) to be shorter in height, profusely branched, small-seeded, and with light-blue petals and brownish seed colours.

Moreover, tall and late maturing types of linseed accessions were initially collected from higher altitudes of Ethiopia and they were found susceptible to lodging (Getinet *et al.*, 1987). Some of the accessions were collected from sites ranging between 1680 meters above sea level in western Ethiopia to 3430 m in northern Ethiopia. The Ethiopian collections of linseed were reported (Adefris *et al.*, 1992) to possess tremendous variability for different characters, such as seed and oil yields, tolerance to biotic (rusts, wilt and pasmo diseases) and abiotic (frost and drought) stresses. Hence, they entail ample opportunities and prospects for the genetic improvement of the crop through either direct selection from the collections or following trait recombination via hybridisation of the desirable parents.

The phenotypic and genotypic coefficients of variability were high for yield/plant, bolls/plant, secondary branches/plant, plant height and days to flowering (Table 4.3). Singh (1980) also found high genetic variability for plant height, boll/plant and branches/plant. Likewise, Mirza *et al.* (1996) and Khorgade and Pillai (1994) observed high phenotypic and genotypic coefficients of variation for boll/plant and plant height. In fact, plant height and primary branches/plant were reported (Jeswani *et al.*, 1970) to contribute maximum divergence and they were regarded as the main characters used to differentiate oil-type linseed from the fibre-type flax. Many other recent reports (Mahto and Mahto, 1998; Mishra and Yadav, 1999; Yadav and Gupta, 1999) also found high genetic coefficients of variation for bolls/plant, primary and secondary branches/plant, 1000-seed weight and seed yield/plant. However, these genetic coefficients of variation should be studied together with estimates of heritability and genetic advance to acquire the best prediction of genetic progress from selection.

The broad sense heritability was estimated to range between 67.38 and 91.38%. Higher percentages (76-91%) of heritability were recorded for days to 50% flowering, plant height, yield/plant, secondary branches/plant, 1000-seed weight and bolls/plant (Table 4.3). Patil *et al.* (1981) also found maximum heritability values (80-89%) for days to 50% flowering and plant height in both F_1 and F_2 generations of linseed. Similarly, high heritability values were also reported (Singh, 1984; Singh *et al.*, 1995; Mirza *et al.*,

1996; Mahto and Mahto, 1998) in linseed for plant height, days to 50% flowering and maturity, yield/plant and 1000-seed weight. Since values of broad-sense heritability are prone to changes with the environment and material (Patil *et al.*, 1981), selections would be more effective for parameters that have shown considerable stability over generations and environments. Moreover, Ceccarelli (1996) and vom Brocke *et al.* (2002) reported that plant breeding activities, which have been conducted under favourable environments showed more selection gains owing to their high heritability and large genotypic variance.

Relatively higher values of genetic advance were estimated for yield/plant, yield (g/m^2), bolls/plant, secondary branches/plant and plant height. Moreover, 1000-seed weight, primary branches/plant and days to 50% flowering showed relatively higher expected genetic gains than the remaining traits. Hence, these results indicate the possibility of obtaining good breeding progress by focusing on these characters in the future breeding efforts. However, the high genetic advance of total yield was primarily attributed to its high genetic coefficient of variation rather than its heritability (Table 4.3). Since environment often influences characters like yield and its major components that are mostly polygenic and have complex inheritance, few gains are expected if selection is predominantly based on such quantitative traits. Johnson *et al.* (1955) suggested that heritability estimates along with genetic advance would be more useful in predicting yield under phenotypic selection than heritability values alone. In the present study, yield/plant, bolls/plant, days to 50% flowering, secondary branches/plant and plant height showed relatively high genotypic coefficients of variability, heritability and genetic advance. Therefore, these traits need to be given more emphasis in phenotypic selections. Mahto and Mahto (1998) and Mishra and Yadav (1999) also found higher heritability along with high genetic advance for days to 50% flowering, yield/plant, plant height and bolls/plant, indicating the predominance of additive gene actions in the expression of these traits. The additive component of genetic variance could not be separated from the variance among accessions that may have led to an overestimation of the genetic gain, especially for traits with a relatively high measure of dominance (vom Brocke *et al.*, 2002).

In general, the current study shows the existence of a broad range of genetic variability in the tested collections for progeny based selection. This variability was also confirmed by the analysis of principal components (Table 4.4) that has explained the overall diversity by 12 eigenvectors. The first eight eigenvectors accounted for about 75.65% of the total variability among the tested accessions. The 3rd eigenvector, which explained 13.72% of the entire variability, was largely because of differences in bolls/plant, primary and secondary branches/plant, yield/plant and powdery mildew score. The principal component analysis showed that the main contributing characters were evenly distributed among the evaluated characters. However, bolls/plant, disease scores, yields/plant, days flowering and branches/plant were the most useful in distinguishing the tested accessions. Kebebew *et al.* (1999) also indicated that the principal component analysis grouped 20 variables of tef (*Eragrostis tef*) into 19 components that accounted for the whole variability, where the first 10 eigenvectors explained about 90% of the entire variance.

The presence of wide diversity among the 60 accessions was further confirmed by the cluster analysis that grouped them into nine classes (Table 4.5 and Fig. 4.1) based on the measurements of 12 agro-morphological characters. The clustering pattern indicated that accessions in clusters VII to IX were genetically more divergent from the other collections for they formed single-genotyped clusters. Hence, they could be used in the crossing programs to develop more productive cultivars. This method of clustering the germplasm collections can also be used in the elimination of duplicate and genetically redundant accessions along with other relevant information and documents of the germplasm (Greene and Pederson, 1996). The study of these authors indicated that accession duplication not only increased the cost of storing, handling and regenerating duplicate accessions but also decreased efficiency of evaluating collections. Hence, they recommended the elimination of duplicates as an effective way of reducing germplasm maintenance costs without losing valuable genetic resources.

4.6 Conclusions

The broad spectrum of variability observed among these collections of linseed for different characters suggests better possibilities for genetic improvement of the crop through selection and cross breeding. The wide ranges in days to maturity can offer great flexibilities for the development of suitable varieties for the various agro-ecological zones of Ethiopia. Different cultivars can be developed for specific agro-ecological regions to make use of the available potentials of both the germplasm and the environment. The current analysis of diversity generally implies the presence of a wealth of genetic diversity for different characters that need be exploited with more targeted exploration and investigation. Thus, further collection should be continued, covering remote areas and focusing on regions that are affected by recurrent droughts. The collection mission can also be easier and more fruitful if carried out as joint activity with the Ministry of Agriculture and the farmers who have good access and means in the collection areas. Further diversity analyses are also needed, applying the evolving DNA markers and other modern tools for effective and efficient identification and utilization of the germplasm.

CHAPTER 5

EVALUATION OF GENETIC DIVERSITY IN LINSEED ACCESSIONS UNDER DIFFERENT ENVIRONMENTS

5.1 Abstract

Sixty accessions of linseed mainly collected from Ethiopia were studied in a randomised complete block design with three replications to assess their genetic diversity, broad sense heritability and genetic advance under four different environments. The combined analysis of variance was highly significant ($P < 0.01$) for 10 quantitative traits, indicating wide ranges of genetic diversity among the accessions. Broad sense heritability and genetic advance were higher for multi-environment tests than the single one, and estimates of both parameters showed maximum values for plant height and days to flowering. Principal component and cluster analyses displayed secondary branches/plant, plant height, days to 50% flowering and yield/plant to play significant roles in differentiating the accessions. These analyses also showed considerable environmental influences in the expression of genetic variability. Better expression of genetic parameters was noted under Holeta field conditions than in glasshouses; thus, similar future evaluation and improvement activities should be continued under such target environments. The cluster analysis grouped the accessions into nine classes based on mean performance of the 10 characters rather than collection areas. The integrated analyses of genetic and environment factors appeared to be a better strategy of germplasm evaluation and improvement to make use of the available resources to their best potential.

5.2 Introduction

According to Esquinas-Alcazar (1993), plant genetic resources represent both the basis for agricultural development and a reservoir of genetic diversity that acts as a buffer against environmental changes. The erosion of these resources threatens world food security and social stability. The need to conserve, evaluate and utilize them as a

safeguard against an unpredictable future is thus well recognized (Frankel, 1989). Genetic resources are limited and perishable natural resources that provide raw materials of genes which, when used and combined in the proper manner, produce new and better varieties with desirable traits, like resistance to diseases, environmental stresses and of high productivity. In order to use the genetic resources with maximum efficiency, their genetic diversity must be evaluated starting from collection to the point where it never ends (Esquinas-Alcazar, 1993). Evaluation can be done anytime, depending on needs and available resources, and it can deal with one or several possible aspects of agronomical, morphological, pathological, cytological, biochemical, etc.

Proper characterization and evaluation of germplasm collections are important components in effective management of genetic resources (Frankel, 1989; Blakeney, 2002) by eliminating unnecessary duplications (Greene and Pederson, 1996). Accurate identification of genotypes or varieties is also very useful throughout the processes of breeding, starting from initial parent selection to the final utilization of cultivars in production schemes. Morphological or phenotypic descriptors have long been used to distinguish one accession from the other. These types of agronomical characterization provide useful information to the users, though they are subjected to environmental influences and thus necessitate studies across environments. Atlin *et al.* (2001) indicated that traits with low heritability could only be precisely evaluated in trials replicated over sites and seasons.

Linseed (*Linum usitatissimum* L.) is one of the oldest annual plants grown by humans for food, oil and fibre (Simmonds, 1976; Lay and Dybing, 1989; Mansby *et al.*, 2000). Ancient Egyptians, Babylonians, Phoenicians and Romanians have appreciated the long-stemmed flax types since antiquity for their strong linen clothes, while the shorter oil-types (linseed) were used in the embalming process of mummies in antique Egypt (Lay and Dybing, 1989). Linseed has also been used as a food during Greek and Roman eras, a tradition that has continued in India, China and Ethiopia (Seegler, 1983; Hiruy, 1987; Lay and Dybing, 1989). Linseed has been the second most important oil crop in the highlands of Ethiopia (Seegler, 1983; Hiruy, 1987; Adugna, 2000). This old crop is now

being revitalized, and is now designated as one of the healthy foods for the 21st century (Cunnane and Thompson, 1995; Flax Council of Canada, 1997; Reinhardt-Martin, 2001). According to Cunnane *et al.* (1995), linseed could be the nutraceutical food of the 21st century because of its multiple health benefits. It has beneficial effects in the prevention of cancer, coronary heart disease, and sudden death from arrhythmia (Flax Council of Canada, 1997, 2001; Reinhardt-Martin, 2001). Linseed is a rich source of omega-3 fatty acids, plant lignans and soluble fibre that offer several health benefits (Carter, 1993; Payne, 2000).

Since linseed has been such an important crop, it is of great necessity to conserve its germplasm as widely possible for future utilization in the breeding programs. In order to maintain and exploit these genetic resources efficiently, an understanding of the amount and distribution of genetic variation within and among accessions is very important. Moreover, plant breeders have consistently emphasized the use of diverse genotypes as a significant factor contributing to high yielding hybrids. However, data in terms of genetic distance between genotypes showing the impact of environment in the expression of genetic diversity are lacking in linseed. Therefore, an effort has been made here to study the role of environment in the genetic differentiation of linseed accessions under different environments. The study can also help the breeders to identify the most suitable environments under which diversity analysis should be conducted to acquire a more realistic picture of genetic divergence. Obviously, quantitative characters that are controlled by a number of genes with small effects are more influenced by the environment than qualitative characters (Poehlman, 1987). The variation in a quantitative character is specified by the phenotypic variance, which includes genetic and environmental components and a genotype-environmental interaction, GEI (Romagosa and Fox, 1993). GEI results because individual genotypes differ in their responses to variations in soil fertility, soil moisture, temperature, daylength, light intensity, humidity, disease, cultural practices, or other environmental factors (Poehlman, 1987; Basford and Cooper, 1998).

According to Romagosa and Fox (1993), GEI reduces association between phenotypic and genotypic values, and may cause selections from one environment to perform poorly in another, forcing plant breeders to examine genotypic adaptation. Its measurement is also important to determine an optimum breeding strategy for releasing genotypes with adequate adaptation to their target environments. Such a study of GEI is especially relevant for countries, like Ethiopia that has very diversified agro-ecologies. According to the recent classification of the Ethiopian Ministry of Agriculture, Ethiopia was grouped into 18 major- and 49 sub-agroecologies (MoA, 1998; Adugna, 2000). Under such conditions the breeders should be able to select desirable cultivars without losing valuable germplasm and other vital resources. Hence, agro-ecological diversity could complicate breeding and testing of improved varieties with adequate adaptation, and it could also permit identification of extreme environmental conditions that might offer selection pressure from different stresses. The objectives of this study were: to investigate the extent of diversity among 60 accessions of linseed under different environments; to estimate heritability (broad sense) and genetic gains of the traits, and to identify and cluster the accessions based on their genetic distances in variable environments for effective utilizations in the future breeding programs. The intension was, therefore, by evaluating and broadening the genetic resources, more productive genotypes could be developed and strengthened for the of future improvement and developments efforts on sustainable basis.

5.3 Materials and Methods

Plant materials

The accessions used in this study were selectively taken from the Highland Oil Crops Research Program of Holeta Research Centre in the Ethiopian Agricultural Research Organization to represent the apparent diversity of linseed germplasm in the country. The accessions have been collected by the Plant Genetic Resource Centre of Ethiopia (now Institute of Biodiversity Conservation and Research, IBCR) in collaboration with the Highland Oil Crops Research Program since the early 1970s. The germplasm collections are preserved at the IBCR and some of their duplicates are also kept at Holeta for research purposes. Totally 60 accessions including four released varieties

(Chilalo, CI-1525, CI-1652 and Belay 96) were studied for their variability and genetic divergence. Belay 96 variety was developed and released in 1996 out of a crossing program undertaken between two locally well-adapted and disease resistant collections at Holeta Research Centre. Fifty-three accessions were collected from different areas of Ethiopia and Eritrea (Figure 3.1), the remaining seven being introductions from Europe, USA and Canada (Table 3.1).

Experimental sites and methods

Glasshouse

The study at Bloemfontein was conducted in the glasshouses of the Agronomy Department during the year 2000 and in that of Plant Breeding Department in 2001 at the University of the Free State (latitude 29° 6' S, longitude 26° 18' E, altitude 1351 m above sea level) in South Africa. The former glasshouse was warmer by about 10°C (average) than the latter, which had a maximum temperature of up to 25°C during the daytime. The minimum night temperature was in the range of 8 to 13°C. Daylight hours ranged from 10.1 in June to 13.9 in December, and April to September had relatively shorter 11.3-11.8 hours of daylight. The 60 accessions were evaluated in pots (2.5 litres) that were laid out in randomised complete block design with three replications. Eight seeds were sown at a depth of about 2.5 cm and thinned to five plants per pot three weeks after planting. The seeds were sown in early September during the first year and in mid June during the second year. A mixture of nitrogen and phosphorus fertilizers of 3 g per pot was applied to the red sandy soil of the Free State province at sowing, 50% flowering and seed filling stages. The plants were irrigated almost every day from planting to maturity.

Field experiment

The field trial was conducted at Holeta Research Centre (latitude 9° 3' N, longitude 38° 30' E, altitude 2400 m above sea level) during the years 2000 and 2001 main cropping seasons in Ethiopia. Monthly temperature ranged between 2-22°C during the growing seasons (June-November), while total annual rainfall varied between 900-

1000 mm per year during the two growing years. The collections were evaluated in two rows (5 m long spaced at 20 cm) that were laid out in randomised complete block design with three replications. The seeds were planted at the seeding depth of about 2.5 cm on June 26 during both years. The recommended fertilizers of both nitrogen (N) in the form of urea and phosphorus (P_2O_5) in the form of diammonium phosphate (DAP) were manually drilled and incorporated with the soil both at a rate of 23 kg/ha at planting. The seeds were also drilled by hand at a rate of 25 kg/ha. Weeds were controlled by hand weeding and no herbicide or insecticide was applied. The plants of each accession was separately harvested and dried for about 15 to 30 days, threshed and cleaned manually. Seed yield data were taken at about 8% seed moisture level.

Data recording

Data on 10 agronomical and morphological characters (Table 5.3) were collected from three and five randomly selected plants in glasshouses and in the field, respectively and their means were recoded for all observations. Except for days to flower initiation, 50% flowering and similar observations, most of the data were recorded at maturity and thereafter. Days to flowering and maturity represent for the number of days from sowing to 50% blooming and physiological ripening, respectively. The unit of measurement for plant height was centimetres (cm), while that of seed yield and 1000-seed weight was in grams (g). In general, most of the evaluation was undertaken based on the major phenological and morphological quantitative descriptors (Diederichsen 2001) that are reported to display high heritability values.

Statistical analysis

The mean values of all sampled observations for the 10 quantitative characters were analysed for their variance and significance using AGROBASE 98 software (Agronomix Software, Inc., 1998). Average of data over the two years were used in defining some basic statistics and were also used for the analyses of principal components and clustering by which classification of the accessions was investigated. In employing the analyses of both principal components and clusters, the 60 accessions were considered as operational taxonomic units and they were represented

by rows and 10 characters taken as variables by columns. Both the principal component and cluster analyses were performed by using the Number Cruncher Statistical System, NCSS 2000 (Hintze, 1998) statistical system for Windows. In the process of hierarchical clustering method, a group average (unweighted pair-group), Manhatan distance and standard deviation scaling type were employed

Estimation of genetic parameters were done to determine the genetic variability among accessions and to display the influence of environmental effects on various characters. The variance components were based on both separate and combined analyses and they were used in partitioning the total variability into its genotypic and environmental factors, adapting the relevant formulae suggested (Singh and Chaudhary, 1977; Fehr, 1987; Atlin *et al.*, 2000; 2001). Accordingly, genotypic and phenotypic components of variance were done as follows: (a) Genotypic variance, GV = MSa - (MSal/l - MSay/y - MSaly/ly - MSe/rly), where MSa = Mean square of accessions, MSal = Mean square of accession by location interaction; MSay = Mean square of accession x year interaction; MSaly = Mean square of accession x location x year interaction; MSe = Mean square of error, l = number of locations; y = number of years; r = number of replications; (b) Phenotypic variance, PV = GV + (MSal/l + MSay/y + Msaly/ly + MSe/rly); (c) Heritability (broad sense), H = GV/PV x 100; (d) Genetic advance, GA = H x PS x K, where PS = phenotypic standard deviation ($\sqrt{PV}$), and K is selection differential at 5 percent selection intensity = 2.06; GA% (as % of mean) = GA/grand mean x 100.

5.4 Results

The mean squares for the combined analysis of variance across years and locations are presented in Table 5.1. Variability among the accessions was highly significant ($P < 0.01$) for all 10 characters, indicating the existence of a wide range of diversity between the tested collections of linseed. Of the entire sources of variation, a large proportion was attributed to the differences in the accessions, confirming their vast genetic diversity. Seasonal variability was also highly significant nearly for all traits due mainly to the variation in edaphic and climatic factors. Variations for the two

locations were also significant for all traits except for seed yield and 1000-seed weight that showed relatively stable performance across environments. Nonetheless, most of the 10 characters showed highly significant ($P < 0.01$) difference across the four environments, indicating the differential responses of the accessions over years and locations. The interaction of accession x location was significant for seven characters, while accession x year was significant for four traits. Similarly, accession x year x location interaction was highly significant ($P < 0.05$) for three characters namely, plant height, days to 50% flowering and days to maturity (Table 5.1). In fact, much of the variability was due to these three characters; followed by secondary branches/plant, seed yields and 1000-seed weight. Thus, the three traits deserve serious consideration in the breeding programs.

The range and mean performance of the accessions under the glasshouse and field conditions (Table 5.2) showed wide ranges of variability among the accessions across the environments, indicating the prevalence of genetic diversity among the accessions and their differential responses to variable environments. For example, plant height varied from its minimum of 36 cm in the year 2000 to the maximum of 122 cm in 2001 at Bloemfontein. Similar trends were also observed for days to 50% flowering and days to maturity.

Table 5.1. Mean squares of the combined analysis of variance for 10 measured traits of 60 linseed accessions evaluated across four environments (UFS and HRC in 2000 and 2001 years)

Source	Df	Traits									
		PH	DF	DM	PB/P	SB/P	B/P	S/B	SY/P	TSY	TSW
Year	1	29594**	42504**	52275**	3.70*	48.57**	130.05*	8.45*	1.49**	776970**	287.94**
Location (L)	1	984.67**	8750**	51461**	2.22*	75.40**	11297**	62.42**	0.12	303.65	6.21
Y x L	1	97767**	48478**	104040**	11.25**	1.70	1105.09**	6.42*	0.08	314.66	287.94**
Entry (E)	59	783.88**	371.68**	193.44**	1.03**	4.67**	65.50**	2.78**	0.18**	3807**	5.41**
E x Y	59	83.27**	29.62	84.19**	0.29	0.87	19.53	0.61	0.07*	2928**	1.49
E x L	59	147.19**	80.72**	67.95**	0.71*	4.43**	32.75*	1.58	0.07	2301**	2.46
E x Y x L	59	165.11**	47.55**	101.71**	0.53	1.02	20.72	0.61	0.06	1125	2.30
Rep (Y x L)	8	1929.60	39.68	423.63**	5.11	35.26**	356.76**	4.61**	0.30**	29223**	79.82**
Residual	472	49.98	27.08	54.20	0.47	1.89	23.84	1.54	0.05	1079.80	1.97

*, ** = Significant at $P < 0.05$, 0.01, respectively; PH = Plant height; DF = Days to 50% flowering; DM = Days to maturity; PB/P = Primary branches/plant; SB/P = Secondary branches/plant; B/P = Bolls/plant; S/B = Seeds/boll; SY/P = Seed yield/plant; TSY = Total Seed yield (g/m^2); TSW = Thousand seed weight.

This vast variability was largely caused by the differences in temperature and sunshine hours in the two glasshouses where the study was conducted, as described in the section of materials and methods. On the other hand, the number of primary branches/plant was nearly consistent across the environments, unlike secondary branches/plant and bolls/plant that varied from four to ten and six to 35, respectively (Table 5.2). Seed yield/plant also ranged from 0.40 g at Holeta to 3.36 g at Bloemfontein in 2000, while 1000-seed weight varied between 3 g and 12 g at Holeta and Bloemfontein, respectively. These results generally show that the performance of the accessions varied tremendously from one environment to the other. These indicate the differential impact of environments on the gene expression of accessions that ultimately affected their phenotype expression (Cooper and Hammer, 1996). The data also revealed the existence of considerable divergence among the collections for different traits.

The estimates of broad sense heritability for the 10 characters across environments are given in Table 5.3. Heritability values were generally higher for the combined results of multi-environment tests than for a single environment. For example, the highest range of heritability (21-81%) was recorded for the combined results of four environments (Bloemfontein-Holeta, 2000-2001) in contrast to the lowest range (1-22%) of Holeta in 2001 (Table 5.3). This result agrees with the report of Atlin *et al.* (2001), which emphasized that broad sense heritability could be increased by improving the precision with which genotypes are evaluated in selection through testing the genotypes over multiple environments.

The highest heritability value of 88.81% was recorded for days to 50% flowering at Holeta in 2000, and this trait also had the highest estimates across the tested environments (Table 5.3). In the same manner, plant height exhibited higher values of heritability across the tested environments. However, some of the remaining characters, such as seed yield/plant, bolls/plant and seeds/boll showed fluctuating values across the environments, indicating the differential expressions of the quantitative traits across the test environments (Poehlman, 1987; Atlin *et al.*, 2001).

Table 5.2. Range and mean (bold) of characters for 60 linseed accessions evaluated in different environments (U = UFS; H = HRC; '00 = 2000; '01 = 2001)

Characters	Environments						
	U '00	U '01	U '00-01	H '00	H '01	H '00-01	UH '00-01
Plant height (cm)	36-71	61-122	52-94	78-97	54-76	58-86	56-88
	53.00	**89.00**	**71.40**	**74.15**	**63.67**	**68.91**	**70.08**
Days to flowering	49-84	79-111	66-96	72-103	75-94	74-97	70-92
	62.70	**95.00**	**78.70**	**86.08**	**85.03**	**85.56**	**82.07**
Days to maturity	98-134	142-168	123-148	147-172	141-156	146-162	135-153
	114.60	**156.00**	**135.20**	**155.55**	**148.55**	**152.05**	**144.00**
P*. branches/plant	3-5	3-6	3-5	3-6	3-5	3-6	3-5
	4.00	**4.25**	**4.23**	**4.38**	**4.13**	**4.26**	**4.20**
S. branches/plant	4-10	5-8	4-9	4-9	4-9	4-9	4-8
	5.50	**6.17**	**5.84**	**6.28**	**6.70**	**6.49**	**6.17**
Bolls/plant	6-21	7-23	7-20	13-35	14-27	15-31	13-25
	11.80	**15.08**	**13.67**	**22.17**	**20.54**	**21.36**	**17.40**
Seeds/boll	8-10	8-10	8-10	6-10	7-11	6-10	7-10
	9.40	**9.43**	**9.40**	**8.61**	**9.02**	**8.81**	**9.11**
Yield/plant (g)	1.66-3.36	1.51-3.19	1.67-3.00	0.40-1.33	0.40-1.13	0.48-1.22	0.51-1.08
	2.29	**2.37**	**2.33**	**0.63**	**0.75**	**0.69**	**0.70**
1000-seed wt. (g)	5-8	7-12	7-10	3-6	3-6	3-6	5-8
	6.85	**9.37**	**8.11**	**4.14**	**4.15**	**4.14**	**6.12**

* P = Primary; S = Secondary

Table 5.3. Heritability estimates (%) for 10 traits of 60 linseed accessions evaluated in different environments (U = UFS; H = HRC; '00 = 2000; 01 = '01 = 2001)

	Environments						
Character	U '00	U '01	U '00-01	H '00	H '01	H '00-01	UH '00-1
Plant height	71.21	65.51	83.29	82.56	12.17	85.21	78.93
Days to flowering	70.24	50.79	85.43	88.81	18.65	87.48	80.98
Days to maturity	25.44	24.72	38.93	42.18	5.15	61.73	37.58
Primary braches/plant	14.29	7.33	41.41	15.46	5.88	66.95	21.04
Sec. branches/plant	27.77	9.37	73.46	7.57	10.55	52.91	27.91
Bolls/plant	22.88	4.24	44.34	22.09	1.06	59.94	38.59
Seeds/boll	20.16	19.44	80.37	6.76	10.95	31.31	29.50
Seed yield/plant	20.16	11.04	15.60	69.23	4.55	3.92	44.44
Total seed yield	25.28	11.37	16.23	44.58	10.41	57.93	57.93
1000-seed weight	63.36	0.03	33.56	7.44	21.65	61.00	37.22

Table 5.4. Expected genetic advance (as percent of mean) of 60 linseed accessions evaluated in different environments (U = UFS; H = Holeta; '00 = 2000; 01 = '01 = 2001)

	Environments						
Character	U '00	U '01	U '00-01	H '00	H '01	H '00-01	UH '00-1
Plant height	22.81	24.97	24.26	24.16	3.28	47.49	65.43
Days to flowering	16.31	10.53	14.53	15.82	2.68	31.03	39.37
Days to maturity	5.06	3.14	3.16	3.9	1.86	9.82	9.02
P*. braches/plant	3.55	2.82	6.69	6.17	2.82	36.05	17.32
S. branches/plant	14.32	3.74	21.34	4.2	1.34	56.13	24.84
Bolls/plant	20.37	3.25	16.70	12.85	1.70	57.98	47.09
Seeds/boll	2.84	2.55	7.44	2.19	0.89	28.58	19.04
Seed yield/plant	11.98	6.67	3.80	35.17	4.00	94.37	63.01
Total seed yield	11.77	6.87	3.97	29.49	5.25	70.30	19.67
1000-seed weight	15.88	5.12	6.02	11.74	8.44	42.56	29.12

* P = Primary; S = Secondary

Although the predicted genetic advance revealed that the highest values of 94.37% (as percent of mean) was recorded for seed yield (g/m^2), followed by that of total seed yield (70.3%) both at Holeta during the combined years of 2000 and 2001 (Table 5.4), their performance was inconsistent across the environments and yield/plant showed very low heritability at Holeta in 2001 (Table 5.3). This result suggests the high liability of yield to environmental influences. The others characters that showed relatively higher genetic gains were bolls/plant, plant height, days to 50% flowering and secondary branches/plant, and most of them displayed consistent performance of larger heritability values across environments. Hence, more genetic progress can be predicted from selection of these traits, as opposed to other traits that displayed fluctuating heritability values across the tested environments.

The principal components analysis that categorized the total variables into 10 independent components (Tables 5.5) showed that the first five eigenvectors accounted for 60.54% of the entire variability among the accessions under the combined analysis of Bloemfontein in 2000-2001, while that of Holeta was 57.66%. However, when the two environments are combined (Bloem-Holeta 2000-2001), the first five eigenvectors were responsible for explaining 54.03% of the total variability. The highest individual variability was explained by the 4^{th}, 2^{nd} and 7^{th} principal components which represented primary branches/plant, days to flowering and seeds/boll, respectively) under Bloemfontein 2000-2001, Holeta 2000-2001 and Bloem-Holeta 2000-2001 environments, respectively (Table 5.5). These results also indicate the influences of environments in explaining the variability of the accessions and the need to evaluate them under different environments to obtain more reliable expression of genetic diversity. The combined analysis of both glasshouse and field data showed that secondary branches/plant, plant height, days to 50% flowering and seed yield/plant (data not shown) played significant roles in differentiating the accessions.

Table 5.5. Eigenvectors and eigenvalues of the first 10 principal components for different characters of 60 linseed accessions tested in environments of UFS and HRC, 2000-2001

Environment/eigenvalues	Eigenvectors									
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
UFS 2000-2001										
Eigenvalue	1.01	0.95	1.02	2.07	1.01	1.02	0.99	0.96	0.98	0.00
Indiv. variation explained (%)	10.09	9.49	10.17	20.72	10.07	10.23	9.92	9.55	9.76	0.00
Cumulative explained (%)	10.09	19.58	29.75	50.47	60.54	70.78	80.69	90.24	100.00	100.00
HRC 2000-2001										
Eigenvalue	1.21	1.50	1.01	1.02	1.03	1.00	1.09	1.14	0.56	0.44
Indiv. variation explained (%)	12.14	14.98	10.09	10.15	10.31	9.98	10.91	11.43	5.62	4.40
Cumulative explained (%)	12.14	27.11	37.20	47.36	57.66	67.64	78.55	89.98	98.95	100.00
UFS-HRC 2000-2001										
Eigenvalue	1.21	1.11	1.03	1.03	1.02	1.14	1.61	0.99	0.19	0.68
Indiv. variation explained (%)	12.09	11.11	10.33	10.29	10.22	11.36	16.12	9.89	6.75	1.85
Cumulative explained (%)	12.09	23.20	33.52	43.82	54.03	65.40	81.51	91.40	93.25	100.00

Note: PC = Principal components

Table 5.6. Number of accessions grouped in different clusters for 60 linseed accessions tested across different environments (U = UFS; H = HRC; '00 = 2000; 01 = '01 = 2001)

Cluster	Environment						
	U'00	U'01	U'00-01	H'00	H'01	H'00-01	UH'00-01
I	3	3	3	2	2	3	2
II	6	3	9	7	2	5	34
III	4	23	2	3	10	5	3
IV	2	29	41	34	14	3	7
V	41	1	1	2	27	2	7
VI	1	1	1	10	1	14	4
VII	1	-*	1	1	1	25	1
VIII	1	-	1	1	1	1	1
IX	1	-	1	-	1	1	1
X	-	-	-	-	1	1	-

* - = nil

Table 5.7. Cluster summary of 60 linseed accessions tested at UFS and HRC, 2000-2001

Cluster	No.of accessions	Name of accessions and their collection areas
I	2	Omega (USA), N1266 (Canada)
II	34	10002, 10104, 10109, 10111, 10119, 10179, 10185, 10197, 10162, 10169 and 10204 (Shewa), 10005 and 10120 (Sidamo), 10159 (Gonder), 10026, 10072, 10073 and 10176 (Gojam), 10085 (Hararge); 10039 (Eritrea), 10041 (Bale), 10046 and 10138 (Welega), 10144 (Welo), 10061 Gamo-Gofa), 10125 and 10042 (Tigray), 10047 (Ilubabor), 10037 (Sidamo), 10246, 10248, 10252, 10254, 10256 and 10258 (n.a.*)
III	3	10022 (Welo), 10060 (Gonder), 10204 (Shewa)
IV	7	Chilalo (Arsi), CI-1525 and CI-1652 (Europe), Belay 96 (Holeta), 10062 (Bale), M20G, and CDCVG (Canada)
V	7	10010 and 10080 (Hararge), 10068 (Gojam), 10118, 10192, 10235, 10250 (Shewa)
VI	4	10007 (Kefa), 10008 (Gonder), 10260 (n.a.) and CDC1747 (Canada)
VII	1	10006 (Ilubabor)
VIII	1	10236 (Gonder)
IX	1	10064 (Gamo-Gofa)

* n.a = not available or specific collection site could not be traced

Table 5.8. Mean performance of nine clusters of 60 linseed accessions tested at UFS and HRC, 2000-2001

Characters	Clusters								
	I	II	III	IV	V	VI	VII	VIII	IX
Plant height (cm)	68.95	71.75	85.00	81.80	78.50	65.26	67.00	62.00	66.00
Days to flowering	85.63	90.25	84.50	87.80	81.25	85.36	89.00	79.00	79.00
Days to maturity	152.20	153.25	156.00	159.40	151.75	150.33	155.00	155.00	151.00
P*. braches/plant	4.33	4.75	5.00	4.40	3.75	4.36	5.00	3.00	4.00
S. branches/p	6.57	8.25	5.50	6.60	5.50	6.54	6.00	7.00	8.00
Bolls/plant	21.40	24.50	29.00	23.20	20.00	20.95	19.00	18.00	30.00
Seeds/boll	8.88	9.00	9.00	8.80	9.00	8.97	7.00	8.00	7.00
Seed yield/plant (g)	0.70	0.70	1.18	0.94	0.78	0.65	0.58	0.57	0.85
Seed yield (g/m^2)	69.75	81.38	78.50	91.60	84.75	65.49	59.50	39.00	54.50
1000-seed weight (g)	6.62	6.06	6.03	6.90	5.58	5.81	5.62	6.35	5.40

* P = Primary; S = Secondary

The cluster analysis under different environments denotes that grouping was influenced both by separate and combined effects of environments (Table 5.6). Consequently, the 60 accessions were classified into six, eight, nine and 10 clusters under different environments, indicating the importance of evaluating the germplasm collections in more than one environment to acquire more reliable results. All in all, the combined cluster analysis across locations and years summarized the accessions into nine clusters (Tables 5.7 and 5.8). Cluster II consisted of the largest number of 34 accessions in contrast to clusters VII, VIII and IX that had only one accession each being the most divergent from the others. The remaining clusters had two to seven accessions each. Thus, such divergent genotypes could be used in the future breeding programs The mean performance of

characters for the nine clusters (Table 5.8) also showed the wide genetic divergence among the tested accessions.

5.5 Discussion

Reliable information on the extent of genetic diversity in the germplasm of linseed is crucial for efficient and effective management and utilization of its germplasm collections. As demands for linseed products are increasingly growing (Flax Council of Canada, 2001) for their multiple health benefits (Cunnane *et al.*, 1995; Payne, 2000; Reinhardt-Martin, 2001), the studies on its germplasm resources could assure its continued genetic improvement for sustainable productivity in the foreseeable future. According to Ceccarelli (1996), the germplasm pool is the basic element of crop production serving as the source of genes for improving performance in a target environment. To this effect, the current study shows a significant range of variation among the accessions for the studied 10 quantitative traits (Tables 5.1 and 5.2). This demonstrates the existence of good sources of genes for developing better performing varieties.

However, since quantitative characters are influenced by environmental factors and result in high GEI (Poehlman, 1987), it is important to assess genetic diversity of crops across environments. Likewise, since diversity is more concentrated in areas of high climatic and ecological diversity (Hawkes, 1983), for example in Ethiopia, one has to focus more on such areas to capture maximum diversity. Moreover, since the phenotypic performance of any crop is a function of genetic, environment and their interaction (Cooper and Hammer, 1996). The integrated consideration of both genetic and environmental factors could be a better strategy for diversity analysis and crop improvement programs. This could also offer more opportunities to improve the productivity of linseed by manipulating both the genotype and environment aspects simultaneously.

Environment can influence the relative performance of genotypes by resulting in GEI, and the factors of environments contributing to GEI can provide a basis for differentiating

types of environments with major roles. Thus, it is necessary to concentrate on describing and understanding causes of GEI in the crop improvement programs. Cooper and Hammer (1996) indicated that the extent of benefits that can be obtained from crop improvement programs would depend on the diversity of production system, the magnitudes of GEI and the strategies deployed to manage these factors. Atlin *et al.* (2001), stressed that if a wide germplasm base is given more emphasis and the potential benefits of GEI are exploited with knowledge-based crop improvement strategies, the current slow rates of progress in the marginal areas could improve their impacts in the farmers' fields.

Several studies of GEI were reported in linseed (Green, 1986a; Rowland *et al.*, 1988; Oomah *et al.*, 1996), of which few were from Ethiopia (Adugna and Labuschagne, 2002). Adugna and Adefris (1995) reported reversal changes in yield ranks (crossovers) of four linseed varieties at two locations in Ethiopia, indicating the differential responses of the genotypes in different environments. Similarly, Adugna and Labuschagne (2002) reported year x location and location variability as the dominant source of interactions in linseed. They identified three genotypes as more stable, while three others were specifically adapted to certain environments. They also emphasized the need for GEI and stability information, whenever new varieties are proposed for commercial release, clearly indicating their specific and/or general adaptations. Green (1986a) also reported the significant genotype x temperature interaction for 1000-seed weight, oil content and fatty acid composition, indicating temperature-sensitive and stable genotypes of linseed in Australian cultivars. Similar studies undertaken in India indicated the presence of significant variability among the genotypes and environments (Mahto *et al.*, 1996). They reported the stability of varieties for seed yield, days to maturity, *Fusarium oxysporum* f. sp. lini resistance, blight tolerance and unfavourable conditions.

A study on GEI, stability and genetic diversity in linseed for yield and yield attributes under dry-land condition of India indicated significant GEI for branches/plant, plant height, seeds/boll and bolls/plant (Mahto, 1995). He also identified 12 genotypes on the basis of stability and genetic divergence for yield and yield attributes. Likewise, Mahto *et*

al. (1996) studied stability and genetic divergence in linseed under rainfed situation and reported the presence of significant variability among the genotypes for all characters. Mishra and Rai (1993) have reported GEI and stability parameters on seed yield and eight quality traits of 10 linseed varieties and their 45 F_1 hybrids grown under four environments in India. They reported stability for seed yield/plant, oil content, protein content, palmitic acid content, and stearic acid content. Another study undertaken to explore the variation in total flavonoid content (antioxidant metabolite) of linseed at four locations in Canada showed that cultivar, environment and their interaction were highly significant (Oomah *et al.*, 1996). Most of these results show that the main effects, cultivar, location and year were dominant, indicating the significant dependence of cultivars performance on the test environment.

Estimates of broad sense heritability under field conditions at Holeta Research Centre showed relatively higher values than that of glasshouses in South Africa (Table 5.3). Indeed, Holeta and its vicinity has been one of the linseed growing areas in Ethiopia. Consequently, characterization and selection of genetic resources have to be conducted in their target environments (Frankel, 1989; Kresovich and Mcferson, 1992; Atlin *et al.*, 2001), such as at Holeta in Ethiopia to get better pictures of their genetic diversity. Relatively, larger heritability values (> 80%) were recorded for plant height and days to 50% flowering and both characters exhibited maximum values across the combined analysis of the four environments (Table 5.3). Moreover, seed yield, bolls/plant, 1000-seed weight and days to maturity showed the next highest heritability estimates. Similarly, Dayal *et al.* (1975) reported that days to flowering, days to maturity, plant height, 1000-seed weight and yield/plant were highly heritable and additive types of gene action were operative for these traits. The combined analysis of principal components also confirmed that most of these traits played significant roles in discriminating the accessions although their magnitude of explaining variability varied from one environment to the other. The differential expressions of the accessions across environments were noted in this analysis (Table 5.5).

Relatively higher genetic gains were predicted for plant height, days to 50% flowering, secondary branches/plant and bolls/plant across the environments (Table 5.4) and their heritability values were also relatively high, indicating that these characters are less influenced by environmental conditions. Johnson *et al.* (1955) have suggested that heritability values together with genetic advance could be more helpful in predicting gain under phenotypic selection than heritability alone.

Linseed accessions with different phenology, particularly flowering and maturity times within the given environmental variations are needed. For example, early flowering and/or early maturing genotypes (e.g., accessions in clusters V, VI and IX of Table 5.8) are required for drought-prone areas of northern Ethiopia. They are also needed in frost-prone central highlands of linseed-producing regions of Ethiopia. Sheno and Tikur-Inchini districts could be cited as a good example for the case in point. On the other hand, late-maturing accessions (e.g., accessions in clusters III and IV, Table 5.8) are preferred in environments that have longer growing periods, like Holeta, Bekoji and Sinana areas of Ethiopia. Farmers in Holeta vicinity are interested in these high yielding and tall varieties of linseed for their better competing ability with weeds and suitability for manual harvesting. To this effect, genotypes with different phenology can be developed to meet the specific adaptations of a particular environment or socio-economic factors.

In general, the combined analysis across environments displayed larger genetic advances than that of individual environments (Table 5.4). Likewise, Atlin *et al.* (2001) indicated that expected genetic progress from selection could be improved by increasing genetic variance (i.e., using a wide range of germplasm) and by increasing heritability across environments (i.e., by increasing replications, sites and years). This could enable precise measurement of genotypic differences under a given selection protocol. The clustering of accessions in variable environments also verified the differential grouping of the germplasm in response to the test environments, indicating the importance of evaluating germplasm in diverse environments for more reliable results. The combined clustering of the four environments condensed and displayed better classification of the accessions, suggesting its effectiveness in the management of germplasm collections.

5.6 Conclusions

The studied accessions showed significant genetic diversity and they were grouped into nine clusters across the environments, demonstrating the benefits of such multi-environment tests to acquire true pictures of germplasm diversity. Estimates of genetic divergence across environments increased heritability and genetic gains, and thus can improve genetic progress under selection. Significant GEI indicates differential responses of accessions across environments that provide opportunities of exploiting the germplasm for broad and specific adaptability, in accordance with their agronomical and phenological attributes. Since environment, like that of Holeta showed more expression of genetic diversity than that of glasshouses in Bloemfontein, such and similar environments should be continued in future linseed germplasm evaluation and improvement efforts. The joint analysis of genetic and environmental diversity could lead to greater productivity, biodiversity and sustainability of linseed and other crops.

CHAPTER 6

VARIABILITY OF OIL CONTENTS AND FATTY ACIDS IN LINSEED ACCESSIONS

6.1 Abstract

The study was undertaken to assess the variability of oil content, oil yield and fatty acid composition of 60 linseed collections to identify suitable accessions for uses in future breeding and development endeavours. Oil content ranged from 29.13 to 35.88%, while oil yield varied between 1443-3276 g/m^2. This variability indicates the possibility for further improvements. Exotic introductions, specifically those from Canada, such as CDC-VG, revealed higher oil contents than the local collections. Thus, introducing exotic materials should be given more emphasis through germplasm exchange programs. Unsaturated fatty acids were the major components; significantly ($P < 0.01$) varying from 85.89 to 90.58%, while the minor saturated fatty acids were within narrow ranges of 8.43-11.91%. Oleic acid ranged between 14.75-29.33%, linoleic 10.88-16.14% and linolenic acid 47.00-59.06%. The 60 accessions were clustered into six groups based on the performance of oil content, oil yield and linolenic acid, but into 10 classes according to the oil yield and fatty acid composition. Although accessions with low and high linolenic acid were identified, the former variability was inadequate to develop genotypes with less than 2% linolenic acid through conventional crossing and selection methods. Hence, mutation breeding techniques and introduction of exotic lines should be regarded as alternative approaches to acquire linseed genotypes with low linolenic acid.

6.2 Introduction

Linseed (*Linum usitatissimum* L.) oil has been mostly famous for industrial purposes in making varnishes, paints and the likes due to its high linolenic (polyunsaturated) fatty acid, which is known for its fast drying quality because of its auto-oxidative three double bonds (Batta *et al.*, 1985; Green, 1986b). Hence, linseed oil has traditionally been used for industrial purposes and as a drying agent in the manufacture of paints,

varnishes, soaps, printer inks, oilcloth and linoleum tiles (Nichterlein *et al.*, 1988; Rowland *et al.*, 1995). Linolenic readily oxides and produces off-flavour (rancidity). This instability problem has limited the use of linseed oil for edible purposes (Rowland, 1994), particularly for cooking oil quality. On the other hand, linolenic fatty acid was reported as one of the essential fatty acids responsible for numerous health benefits (Carter, 1993; Oomah *et al.*, 1996; Payne, 2000; Reinhardt-Martin, 2001). According to these authors, the apparent health benefits of linolenic acid include anti-hypercholesterolemic and anti-carcinogenic effects and it is essential in the development of brain and retinal tissues of infants. To make use of these benefits, therefore, one has to consume the seeds in various forms of baked or cooked products (Carter, 1993; Reinhardt-Martin, 2001). Indeed, linseed has traditionally been consumed and appreciated for various medicinal values in countries like Ethiopia, India and China since ancient times (Seegler, 1983; Carter, 1993).

On the other hand, in order to convert linseed oil into premium edible types, research efforts have been underway in Australia (Green and Marshall, 1981) and Canada (Rowland, 1994) since the early 1980s. Those efforts have led to the release of *Linola* and *Solin,* low linolenic (< 2%) varieties in both countries (Rowland, 1994). A recent study (Ntiamoah *et al.*, 1995) also revealed the possibility of increasing the palmitic fatty acid of linseed to 30% to use it for the production of margarine, shortening and other fat products. These experiences clearly show the possibilities of expanding market opportunities for linseed by developing cultivars with different fatty acid profiles (Nichterlein *et al.*, 1988).

Linseed has been a traditional crop in Ethiopia and it is the second most important oil crop in the highlands of Ethiopia (Adugna and Labuschagne, 2002). Small-scale farmers have been producing it organically, without applying any chemicals (fertilizers, herbicides, etc.) and with minimum inputs. It has been grown in Ethiopia primarily for food and to generate cash revenues for the farmers either on local markets or by exporting abroad. For food, the seeds are usually roasted, ground and mixed with spices and some water to be served along with different local breads

(Adugna, 2000). It is also consumed in various forms of soups, soft drinks and with porridges, cooked potatoes, etc. Limited amounts of linseed is also locally pressed for its edible oil that are often blended with oils of other oilseeds that have high oil quality, such as noug (*Guizotia abyssinica* CASS) that has about 75% linoleic acid (Getinet and Adefris, 1992). Blending is undertaken to minimize the effects of rancidity and to maintain acceptable taste and flavour of cooking oils.

Ethiopia has been cited as one of the centres of diversity for linseed (Seegler, 1983; Hiruy, 1987). However, little efforts were made to collect, evaluate and utilize the available germplasm, particularly in terms of fats and fatty acid profiles mainly due to lack of laboratory facilities. In this study, therefore, attempts were made to assess the levels of variation in oil content, oil yield and fatty acids existing among the collections of Ethiopia along with a few introductions, and to identify suitable accessions of linseed for uses in future breeding and development programs.

6.3 Materials and methods

Germplasm accessions

The accessions used in this study were selectively taken from the Highland Oil Crops Research Program of Holeta Research Centre in the Ethiopian Agricultural Research Organization to represent the apparent diversity of linseed germplasm in the country. The Institute of Biodiversity Conservation and Research (IBCR) initially collected these accessions in collaboration with the Highland Oil Crops Research Program since the early 1970s (Hiruy, 1987). The germplasm collections are preserved at the IBCR and some of their duplicates are also kept at Holeta for research purposes. Totally 60 accessions, including four released varieties (Chilalo, CI-1525, CI-1652 and Belay 96) were studied for their variability of fats and fatty acids. Belay 96 was developed from a cross made between locally well-adapted and disease resistant collections at Holeta Research Centre and it was released in 1996. Fifty-three accessions were collected from different areas of Ethiopia and Eritrea (Fig. 3.1), the remaining seven being introductions from Europe, USA and Canada (Table 3.1). All the seeds that were analysed for fatty acids were taken from those grown under similar conditions at

Holeta Research Centre (latitude 9° 3' N, longitude 38° 30' E, altitude 2400 m above sea level) during the 1999 main cropping season. These accessions were evaluated in two rows (5 m long spaced at 20 cm = 2 m^2) at a seeding depth of about 2.5 cm sown during the last week of June 1999. The recommended fertilizers of both nitrogen (N) in the form of urea and phosphorus (P_2O_5) in the form of diammonium phosphate (DAP) were manually drilled and incorporated with red-clay soils (eutric Nitosol), both fertilizers at a rate of 23 kg/ha at planting. The seeds were also drilled by hand at a rate of 25 kg/ha. Weeds were controlled by hand weeding about 2-3 times as required. Neither herbicides nor insecticides were applied. The plants of each accession was separately harvested and dried for about 15 to 30 days, threshed and cleaned manually. Seeds were oven dried to about 1% seed moisture level for oil analysis. Oil yield (g/m^2) was estimated by multiplying the mean values of oil content (%) and seed yield (g/m^2) for each accession.

Analyses of oil content and fatty acids

Oil contents were determined by two methods of wide line nuclear magnetic resonance (NMR) and solvent extraction method (SEM). About 25 g of oven-dried seeds were analysed by NMR (Newport Analyser) with reference to a standard of extracted linseed oil at Holeta Research Centre. In South Africa, however, oils were extracted for fatty acid analysis from about 0.5 g of ground seed of linseed, following the organic solvent extraction method of Folch *et al.* (1957) in the Department of Food Science at the University of the Free State (Appendix 1). Fatty acid methyl esters were prepared following the method of Slover and Lanza (1979). Fatty acids were quantified using a Varian GX 3400 flame ionisation gas chromatograph, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 μm ID, 0.2 μm film thickness). Column temperature was 40-230°C (hold 2 minutes; 4°C/min.; hold 10 minutes). Fatty acid methyl esters in hexane (1 μl) were injected into the column using a Varian 8200 CX Autosampler with a split ratio of 100:1. The injection port and detector were both maintained at 250°C. Hydrogen was used, as the carrier gas at 45 psi and nitrogen was the makeup gas. Chromatograms were recorded with Varian Star Chromatography Software. Identification of sample fatty acids was made by

comparing the relative retention times of fatty acid methyl ester peaks from samples with those of standards obtained from SIGMA (Cat. No. 189-19).

Statistical analysis

The mean values of oil content and fatty acid composition were analysed by descriptive statistics, using the Number Cruncher Statistical System, NCSS 2000 (Hintze, 1998) statistical system for Windows. The critical difference (CD) was computed by multiplying the standard error by the tabulated t-values to test the significance levels between the mean values of each accession. If the mean difference between any two accessions is greater than the computed critical difference values, the variability between the accessions is considered significant (Singh and Chaudhary, 1977). The same mean data of each accession was used to perform clustering analysis. In this analysis, the 60 accessions were considered as operational taxonomic units and they were represented by rows and the oil content and fatty acids were taken, as variables by columns. In the process of hierarchical clustering, unweighted pair group method of arithmetic average (UPGMA) and standard deviation scaling type were employed.

6.4 Results

The data of oil content and oil yield for the studied accessions were presented in Table 6.1. The highest mean oil content (35.88%) was obtained from CDC-VG in contrast to the lowest (29.13%) that was recorded from accession 10260. The former was a selection from CDC 1747 (an introduction from Canada), while the latter was collected from Ethiopia (Table 3.1). The other accessions, which had high oil percentage included 10037, 10120, 10159, 10062 and N1266 (Table 6.1). Similarly, the highest oil yield of 3276 g/m^2 was obtained from Belay 96, whereas the lowest of 1443 g/m^2 was recorded from accession 10204 that was collected from Shewa region of Ethiopia. Like Belay 96, all the other three commercial varieties of linseed in Ethiopia (Chilalo, CI-1525 and CI-1652) showed relatively higher oil content and oil yield (Table 6.7) than the remaining accessions, indicating their continued good performance in this regard.

The results of the two oil determining methods (NMR and SEM) revealed that the non-destructive NMR method displayed an average of 32.99% oil content against 31.12% (Table 6.1) of the Folch *et al.* (1957) solvent extraction method. The difference between the two methods was not significant, and the NMR method appeared easier and more effective than the solvent extraction to analyse numerous samples and collections within a short period time. However, if fatty acid compositions are to be quantified we need the solvent extraction method to obtain the oils for fatty acid analysis.

Table 6.1. Oil content (%) and oil yield (g/m^2) of 60 linseed accessions analysed by solvent extraction method (SEM) and nuclear magnetic resonance (NMR), 2001

		Oil content (%)			Oil			Oil content (%)			Oil
No	Accession	SEM	NMR	Mean	Yield	No	Acc.	SEM	NMR	Mean	Yield
1	10002	34.88	32.20	33.54	2398	34	10185	29.70	31.20	30.45	2010
2	10005	30.51	31.90	31.20	2106	35	10192	26.96	32.90	29.93	1991
3	10007	33.98	32.60	33.29	2480	36	10197	29.79	32.70	31.25	2203
4	10008	34.08	33.00	33.54	2482	37	10204	33.55	32.80	33.17	1443
5	10010	27.34	32.40	29.87	2091	38	10006	29.70	32.00	30.85	2175
6	10022	31.65	33.20	32.42	2318	39	10042	31.79	33.80	32.80	2509
7	10026	35.37	32.00	33.68	2425	40	Belay 96	35.96	34.10	35.03	3276
8	10037	33.70	33.90	33.80	2349	41	10047	28.96	31.30	30.13	1718
9	10039	32.70	32.80	32.75	2473	42	10062	32.88	35.10	33.99	2363
10	Chilalo	34.15	35.00	34.58	3026	43	10235	28.53	33.00	30.76	2077
11	10041	33.73	31.80	32.77	1934	44	10236	33.35	33.20	33.28	2080
12	10046	32.15	31.90	32.03	1970	45	10064	32.84	30.00	31.42	1948
13	10060	31.68	34.50	33.09	1886	46	10072	30.61	31.90	31.26	2110
14	10061	28.68	32.90	30.79	1955	47	10073	31.43	33.90	32.67	2483
15	10068	30.25	33.50	31.87	2486	48	10246	30.29	32.20	31.24	2078
16	10080	32.06	31.10	31.58	1737	49	10248	29.92	31.60	30.76	2123
17	10085	29.02	32.60	30.81	2034	50	10250	28.32	31.70	30.01	1756
18	10104	28.21	33.00	30.61	2189	51	10252	30.85	33.00	31.93	2523
19	10109	27.39	32.30	29.84	1820	52	10254	30.28	31.90	31.09	2068
20	CI-1525	34.45	36.80	35.62	2903	53	10256	28.46	31.20	29.83	2103
21	10111	31.15	31.50	31.33	2303	54	10258	32.06	31.90	31.98	2431
22	10118	30.42	32.00	31.21	2232	55	10260	27.27	31.00	29.13	1763
23	10119	31.62	31.40	31.51	2127	56	Omega	29.09	36.00	32.55	2604
24	10120	33.81	33.90	33.85	2522	57	N1266	32.85	34.80	33.82	2317
25	10125	29.47	33.10	31.29	1737	58	M20G	30.94	35.90	33.42	2874
26	10138	28.17	31.70	29.93	2095	59	CDC17	28.91	37.40	33.15	2536
27	10144	29.12	33.90	31.51	2143	60	CDCV	35.86	35.90	35.88	3212
28	10159	33.79	33.90	33.84	2251		Mean	31.12	32.99	32.06	2243
29	10162	28.43	33.70	31.07	2097		S.E. ±	0.32	0.20	0.21	48.00
30	CI-1652	34.69	35.30	35.00	2993		Min.	26.96	30.00	29.13	1443
31	10169	32.58	33.20	32.89	2270		Max.	35.96	37.40	35.88	3276
32	10176	27.15	31.70	29.42	2030		CD 0.05	0.53	0.33	0.35	79.00
33	10179	29.57	32.40	30.98	1952		CD 0.01	0.76	0.48	0.50	114.00

Table 6.2. Cluster distribution[1] of 60 linseed accessions based on their mean oil content and oil yield, 2001

Cluster	No. acc.	Oil (%)	Oil yield (g/m^2)	Name of accessions and their collection areas as shown in the parenthesis
I	2	35.75	3057	CI-1525 (Europe), CDC-VG (Canada)
II	3	34.87	3098	Chilalo (Arsi), Belay 96 (Holeta), CI-1652 (Europe)
III	14	33.53	2315	10002 (Shewa), 10204 (Welo), 10007 (Kefa), 10008, 10060, 10159 and 10236 (Gonder), 10026 (Gojam), 10037 and 10120 (Sidamo), 10062 (Bale), N1266, M20G and CDC-1747 (Canada)
IV	21	31.13	2067	10005 (Sidamo), 10061 and 10064, (Gamo-Gofa), 10080, 10085, (Hararge), 10104, 10111, 10118, 10119, 10179, 10185 and 10197, (Shewa), 10125 (Tigray), 10144 (Welo), 10162 and 10235 (Gonder), 10006 (Ilubabor), 10072 (Gojam), 10246, 10248 and 10254 (n.a.)
V	9	29.79	1930	10047, 10006 (Ilubabor), 10250, 10256 and 10260 (n.a.), 10138 (Welega) 10176 (Gojam), 10010 (Hararge), 10109 (Shewa)
VI	11	32.42	2364	10022 (Welo) 10046 (Welega), 10039 (Eritrea), 10041 (Bale), 10068 and 10073 (Gojam), 10169 (Gonder), 10042 (Tigray), 10252 and 10258 (n.a.), Omega (USA)

[1] UPGMA method, Euclidean distance and standard deviation scale; n.a. = not available

Table 6.3. Fatty acid composition (%) of the 60 linseed accessions analysed at UFS, 2001

Accession	Saturated fatty acids			Unsaturated fatty acids			
	Palmitic (C16:0)	Stearic (C18:0)	Total	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)	Total
10002	6.24	5.01	11.25	25.09	12.04	50.46	87.59
10005	6.27	5.17	11.44	27.50	11.53	48.35	87.38
10007	6.23	5.32	11.55	19.00	13.57	54.44	87.01
10008	6.01	4.84	10.85	23.19	13.14	51.47	87.80
10010	6.57	4.32	10.89	26.59	11.66	49.54	87.79
10022	6.14	5.11	11.25	24.12	12.90	50.38	87.40
10026	5.98	5.19	11.17	21.33	13.22	52.96	87.51
10037	6.12	4.89	11.01	29.33	10.88	47.51	87.72
10039	5.88	5.33	11.21	27.30	12.28	47.78	87.36
Chilalo	6.05	4.36	10.41	27.48	13.75	47.14	88.37
10041	5.78	5.09	10.87	27.97	11.50	48.34	87.81
10046	5.86	4.75	10.61	23.16	13.58	51.57	88.31
10060	6.01	5.35	11.36	25.44	12.94	49.07	87.45
10061	6.53	5.38	11.91	24.42	12.31	49.86	86.59
10068	6.44	5.09	11.53	27.08	12.57	47.00	86.65
10080	5.69	4.79	10.48	24.78	12.45	51.10	88.33
10085	6.54	5.09	11.63	22.43	13.43	50.03	85.89
10104	6.23	4.84	11.07	27.82	12.40	47.35	87.57
10109	6.03	4.52	10.55	20.95	12.97	54.40	88.32
CI-1525	5.96	4.76	10.72	23.55	14.10	50.15	87.80
10111	5.97	5.04	11.01	23.47	12.88	51.45	87.80
10118	6.05	5.08	11.13	26.09	12.45	48.98	87.52
10119	5.92	5.34	11.26	23.98	12.45	50.92	87.35
10120	5.70	5.51	11.21	26.32	11.87	49.32	87.51
10125	5.88	4.85	10.73	21.95	14.32	51.76	88.03
10138	6.12	5.09	11.21	17.63	14.30	55.55	87.48
10144	6.29	4.77	11.06	22.36	13.23	51.99	87.58
10159	5.63	5.31	10.94	20.06	14.39	53.62	88.07
10162	5.73	5.10	10.83	18.87	15.02	54.15	88.04
CI-1652	5.91	4.49	10.40	23.14	14.66	50.75	88.55
10169	6.03	5.19	11.22	22.98	12.72	51.79	87.49
10176	6.41	4.91	11.32	22.05	13.24	52.05	87.34
10179	6.33	4.90	11.23	22.90	13.32	50.18	86.40
10185	5.89	5.00	10.89	21.63	13.53	52.61	87.77
10192	6.36	5.02	11.38	23.04	13.06	51.23	87.33
10197	6.35	4.82	11.17	22.18	13.01	52.29	87.48
10204	6.14	5.70	11.84	22.98	12.78	51.07	86.83
10006	6.31	4.76	11.07	20.99	13.84	52.86	87.69
10042	5.97	4.63	10.60	23.65	13.34	51.19	88.18
Belay 96	5.70	4.51	10.21	22.49	14.42	51.55	88.46
10047	6.14	4.99	11.13	24.98	13.53	49.17	87.68
10062	5.90	5.02	10.92	26.66	12.20	48.93	87.79
10235	6.49	4.94	11.43	23.27	12.96	51.17	87.40
10236	5.65	5.09	10.74	21.81	14.12	52.30	88.23
10064	5.71	4.93	10.64	18.33	15.22	54.73	88.28
10072	6.28	5.51	11.79	24.34	12.49	50.14	86.97
10073	6.47	5.39	11.86	25.11	12.92	48.81	86.84
10246	5.89	4.47	10.36	23.24	13.36	51.88	88.48
10248	5.70	5.09	10.79	23.00	12.70	52.39	88.09
10250	6.24	4.64	10.88	19.33	13.31	55.24	87.88
10252	6.05	4.88	10.93	15.90	13.94	58.05	87.89
10254	6.39	5.02	11.41	16.72	13.95	55.25	85.92
10256	5.98	4.27	10.25	14.76	14.62	59.06	88.44
10258	6.02	4.88	10.90	16.75	13.63	57.48	87.86
10260	6.01	4.84	10.85	17.33	14.19	56.38	87.90
Omega	5.19	3.83	9.02	19.25	15.67	54.98	89.90
N1266	5.57	2.86	8.43	20.29	13.39	56.90	90.58
M20G	5.81	3.54	9.35	16.94	16.14	56.47	89.55
CDC1747	5.67	4.91	10.58	17.73	15.80	54.90	88.43
CDC-VG	5.41	4.21	9.62	20.74	15.27	53.37	89.38
Mean	6.03	4.88	10.91	22.56	13.36	51.86	87.78
S.E. ±	3.79	6.05	8.19	0.44	0.14	0.37	0.11
Minimum	5.19	2.86	8.43	14.76	10.88	47.00	85.89
Maximum	6.57	5.70	11.91	29.33	16.14	59.06	90.58
CD 0.05	6.33	10.11	13.69	0.74	0.23	0.62	0.18
CD 0.01	9.06	14.46	19.57	1.05	0.33	0.88	0.26

Table 6.4. Cluster distribution[1] of 60 linseed accessions based on their fatty acids, 2001

Cluster	No. acc.	Name of accessions and their collection areas in parenthesis
I	2	Omega (USA), CDC-VG (Canada)
II	5	10159, 10162 and 10236 (Gonder), 10064 (Gamo-Gofa), CDC1747 (Canada)
III	8	10007 (Kefa), 10119 (Shewa), 10138 (Welega), 10250, 10252, 10254, 10258 and 10260 (n.a.)
IV	9	10104 and 10118 (Shewa), 10005, 10037 and 10120 (Sidamo), 10041 and 10062 (Bale), 10060 (Gonder), 10039 (Eritrea)
V	31	10002, 10111, 10119, 10179, 10185, 10192 and 10197 (Shewa) Belay 96 (Holeta), 10008, 10060, 10169 and 10235 (Gonder), 10046 (Welega), 10026, 10176, 10072, 10073 (Gojam), 10144, 10022 and 10204 (Welo), 10061 (Gamo-Gofa), 10080 and 10085 (Hararge), 10125 and 10042 (Tigray), CI-1525 and CI-1652 (Europe), 10006 and 10047 (Ilubabor), 10246 and 10248 (n.a.)
VI	1	10010 (Hararge)
VII	1	Chilalo (Arsi)
VIII	1	10256 (n.a)
IX	1	N1266 (Canada)
X	1	M20G (Canada)

[1] UPGMA method, Euclidean distance and standard deviation scale; n.a. = not available

Based on the mean performance of oil contents and oil yields, the 60 accessions were grouped into six clusters (Table 6.2). Cluster I consisted of two exotic genotypes, of which one was the commercial variety CI-1525 and the other CDC-VG, a selection from the Canadian variety, CDC-1747. Both genotypes in cluster I had the highest oil content and reasonably good oil yield. Similarly, cluster II comprised of three licensed cultivars and also displayed the next highest oil content and oil yield (Table 6.2). Cluster III consisted of 14 accessions, of which 11 were local collections and the remaining three were introduced from Canada. Cluster IV had the largest number of 21 accessions, all being local collections from different parts (eastern, western, southern and northern) of Ethiopia. The fifth cluster also had nine accessions collected from different areas of Ethiopia and this cluster was found with the lowest oil content and oil yield. In general, most of the genotypes that gave higher oil contents and oil yields were the exotic types. Of these, CDC-VG was the most promising either for direct release or to be used in the future breeding programs.

The results of fatty acid analysis are presented in Table 6.3. There was a highly significant ($P < 0.01$) difference among the accessions for the unsaturated fatty acids, while the saturated ones showed non-significant differences. In fact, palmitic and stearic fatty acids were within narrow ranges of 5.19-6.57% and 2.86-5.70%, respectively. In this regard, accession N1266 had the lowest saturated fatty acid of 8.43% against its highest unsaturated fatty acid (90.58%). Likewise, accession 10037 showed the highest concentration (29.33%) of oleic, while M20G displayed the highest (16.14%) linoleic acid. Similarly, the highest linolenic (59.06%) was obtained from accession 10256, followed by that of accession 10252 (Table 6.3).

In general, most of the introduced genotypes from Canada showed higher amounts of unsaturated fatty acids and lower saturated fatty acids than the Ethiopian accessions. Wide ranges of variation were noted for unsaturated fatty acids in contrast to that of the saturated ones. Genotypes with a higher concentration of linolenic acid were required largely for various industrial applications, but these days they are being rediscovered as a rich source of essential fatty acids (Carter, 1993; Flax Council of

Canada, 2001), imparting numerous health benefits (Payne, 2000; Reinhardt-Martin, 2001). On the other hand, germplasm collections with higher concentrations of oleic and linoleic fatty acids are also needed for cooking oils with acceptable shelf-lives (Green, 1986b; Rowland, 1994).

Table 6.5. Mean performance of fatty acid profiles for 10 clusters of 60 linseed accessions, 2001

	Saturated fatty acids			Unsaturated fatty acids			
Cluster	C16:0	C18:0	Total	C18:1	C18:2	C18:3	Total
I	5.30	4.02	9.32	20.00	15.47	54.18	89.65
II	5.68	5.07	10.75	19.36	14.91	53.94	88.21
III	6.12	5.00	11.12	18.33	13.67	55.41	87.41
IV	5.99	5.14	11.13	27.16	12.01	48.40	87.57
V	6.13	4.95	11.08	23.22	13.19	51.15	87.56
VI	6.57	4.32	10.89	26.59	11.66	49.54	87.79
VII	6.05	4.36	10.41	27.48	13.75	47.14	88.37
VIII	5.98	4.27	10.25	14.76	14.62	59.06	88.44
IX	5.57	2.86	8.43	20.29	13.39	56.90	90.58
X	5.81	3.54	9.35	16.94	16.14	56.47	89.55

The current accessions were classified into 10 clusters (Table 6.4) based on their fatty acid composition. Cluster I had two accessions, which were introduced from Canada. They were characterized by a lower concentration of saturated fatty acids and higher unsaturated fatty acids (Tables 6.3 and 6.5). Similarly, cluster II consisted of five accessions, while clusters III, IV and V were comprised of eight, nine and 31 accessions, respectively. These latter clusters (III, IV and V) exhibited relatively higher levels of saturated fatty acids and lower unsaturated fatty acids (Table 6.5). Cluster V had the largest number of accessions consisting of both local collections and exotic introductions. This indicates the clustering pattern was not necessarily based on the geographic origins of the accessions. Clusters VI to X each had one accession, indicating wide variability in their fatty acid composition that can be utilized in future breeding programs.

Table 6.6. Cluster distribution[1] of 60 linseed accessions based on their linolenic fatty acid composition, 2001

Cluster	No. Acc.	Mean % linolenic	Name of accessions and their collection areas in parenthesis
I	5	47.36	10037 (Sidamo), 10039 (Eritrea), Chilalo (Arsi), 10068 (Gojam), 10104 (Shewa)
II	9	54.85	10007 (Kefa), 10109 (Shewa), 10138 (Welo), 10162 (Gonder), 10064 (Gamo-Gofa), 10250 and 10254 (n.a.), Omega (USA), CDC1747 (Canada)
III	5	57.06	10252, 10258 and 10260 (n.a.), N1266 and M20G (Canada)
IV	16	49.48	10002, 10118 and 10179 (Shewa), 10005 and 10120 (Sidamo), 10010 and 10085 (Hararge), 10022 (Welo), 10041 and 10062 (Bale), 10060 (Gonder), 10061 (Gamo-Gofa), CI-1525 (Europe), 10047 (Ilubabor), 10072 and 10073 (Gojam)
V	24	51.83	10008, 10159, 10169, 10235 and 10236 (Gonder), 10026 and 10176 (Gojam), 10046 (Welega), 10144 and 10204 (Welo), 10080 (Hararge), 10111, 10119, 10185, 10192 and 10197 (Shewa), Belay 96 (Holeta), 10125 and 10042 (Tigray), CI-1525 (Europe), 10006 (Ilubabor), 10246, 10248 and 10250 (n.a.*)
VI	1	59.06	10256 (n.a.)

[1]UPGMA method, Euclidean distance, standard deviation scale;* n.a. = not available.

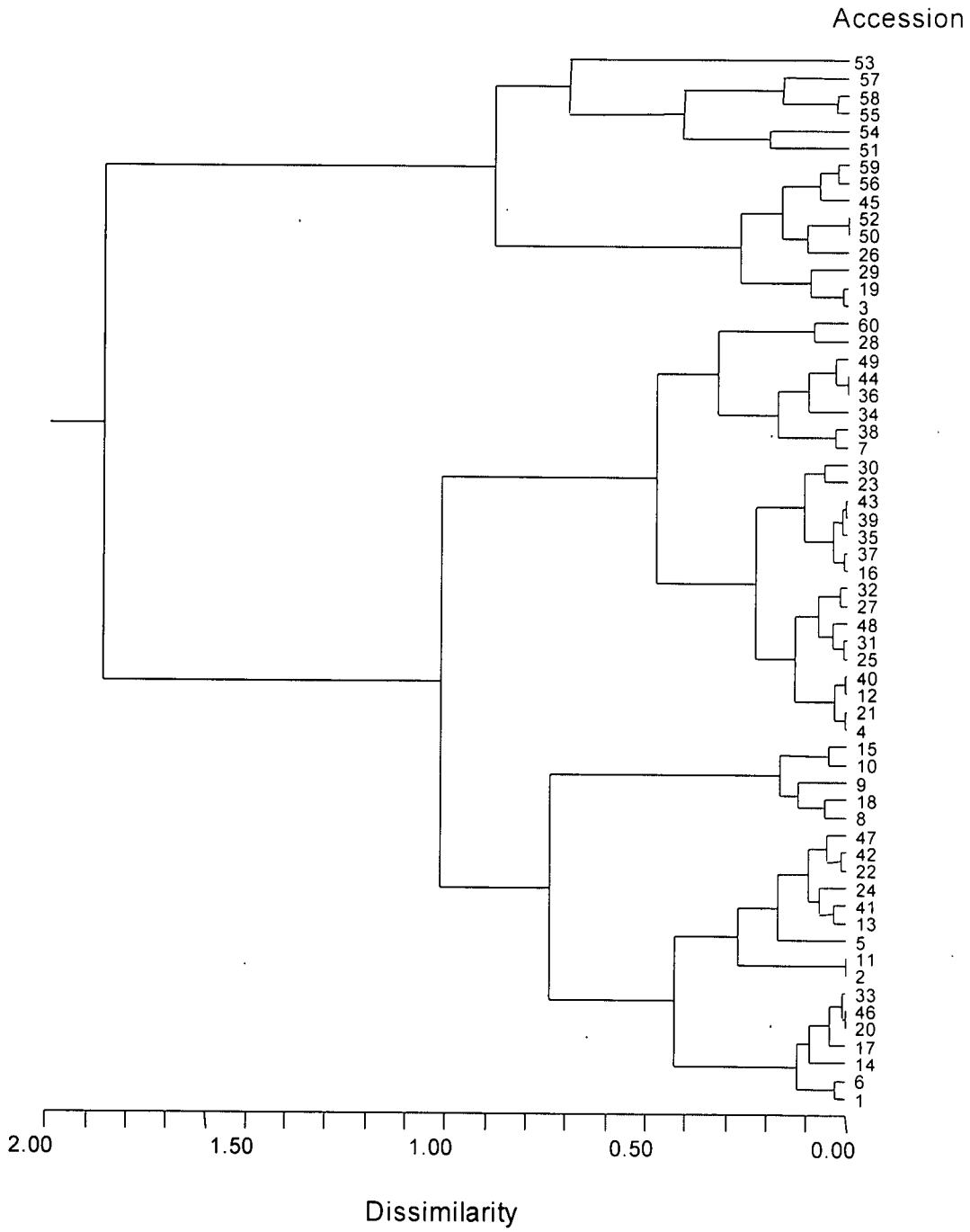


Fig. 6.1. Dendrogram illustrating clusters of 60 linseed accessions (Serial no.) based on their linolenic acids evaluated at UFS in 2001 (cut-off point = 0.5)

Table 6.7. Summary of cluster distribution[1] of 60 linseed accessions based on their mean oil yield, saturated and unsaturated fatty acids, 2001

Cluster	No. acc.	Oil yield	Sat. FA	Unsa FA	Name of accessions and their collection areas as shown in the parenthesis
I	2	2157	11.6	87.1	10060 (Gonder), 10204 (Welo)
II	5	2328	11.0	87.6	10037 and 10120 (Sidamo), 10039 (Eritrea), 10041 (Bale), 10062 (Bale)
III	5	2318	10.4	87.3	10159, 10162 and 10236 (Gonder), 10064 (Gamo-Gofa), CDC-1747 (Canada)
IV	4	3050	10.6	88.4	Chilalo (Arsi), CI-1652 and CI-1525 (Europe), Belay 96 (Holeta)
V	8	2069	10.9	87.7	10109 (Shewa), 10138 (Gojam) 10250, 10252, 10254, 10256, 10258 and 10260 (n.a.)
VI	31	2157	11.2	87.5	10002, 10104, 10111, 10118, 10119, 10179, 10185 100192 and 10197 (Shewa), 10005 (Sidamo), 10007 (Kefa), 10008 and 10169 (Gonder), 10042 and 10125 (Tigray), 10047 (Ilubabor), 10235 (Gonder), 10022 and 10144 (Welo), 10026, 10068, 10176, 10072 and 10073 (Gojam), 10046 (Welega), 10061 (Gamo-Gofa), 10080 and 10085 (Hararge) 10006 (Ilubabor), 10246 and 10248 (n.a.)
VII	2	2739	9.2	89.5	Omega (USA), M20G (Canada)
VIII	1	2091	10.9	87.8	10010 (Hararge)
IX	1	2317	8.43	90.6	N1266 (Canada)
X	1	3212	9.62	89.4	CDC-VG (Canada)

[1] UPGMA method, Euclidean distance and standard deviation scale.

Cluster VI was composed of 10010, an accession collected from Hararge (eastern Ethiopia) and it showed the highest palmitic and lower linoleic fatty acids (Tables 6.3 and 6.5). Similarly, Chilalo, which was clustered in VII, had high oleic acid (27.48%) and low linolenic acid (47.14%). On the other hand, accession 10256 that was categorised in cluster VIII revealed the lowest oleic (14.76%) and the highest linolenic acid (59.06%). This inverse relationship between oleic and linolenic was also reported elsewhere (Batta *et al.*, 1985; Green, 1986b), suggesting the synthesis of oleic, linoleic and linolenic fatty acids in this descending order.

Clustering of the accessions was also undertaken based on the performance of linolenic acid, and the accessions were grouped into six clusters (Table 6.6 and Fig. 6.1). Cluster I consisted of five accessions that had the lowest linolenic acid, while cluster II contained nine accessions, with an average linolenic of 54.85%. Cluster III comprised of five accessions and showed the second highest linolenic acid, next to cluster VI that consisted of only one accession (10256), with the highest linolenic acid (59.06%). Cluster IV had 16 accessions, whereas cluster V consisted of 24 accessions within a narrow range of linolenic (49-52%), as shown in Table 6.6. The accessions grouped in clusters III and VI could be useful for developing cultivars rich in omega-3 fatty acids. Despite this variability, however, the natural variability observed in this study was not good enough for developing high quality cooking oil (i.e., < 2 % linolenic acid) and thus other breeding approaches, such as mutation techniques should be applied, as exemplified in Australia and Canada (Green, 1986b; Rowland *et al.*, 1995).

6.5 Discussion

Increasing the yield of oil per unit area and improving its oil quality for cooking purposes are some of the major breeding objectives of linseed research in Ethiopia. The first objective of increasing oil yield per hectare could be achieved by increasing seed yield/ha and by increasing oil content of the seed. Oil content can be increased by selecting varieties with higher oil contents and also by decreasing the proportion of the seed that is seed coat and protein (Knowles, 1983). There is an inverse relationship

between oil and protein content and between oil and fibre content (Knowles, 1983; Naqvi *et al.*, 1987). Since oil is usually sold at a higher price than the meal, most breeders tend to increase oil at the expense of meal and protein contents.

The mean performance of oil content and oil yield for the investigated accessions were in the wide ranges of about 29-36% and 1443-3276 g/m^2, respectively (Table 6.1). The top oil-yielding accessions were the four commercial varieties, confirming that they are still superior to many local collections. Thus, they could be used as a recurrent parent in the future improvement programs. The next promising accessions include CDC-VG, M20G, 10120, 10042, 10252 and Omega, their oil yield being in the ranges of 2509-3212 g/m^2. Adefris *et al.* (1992) also reported that the Ethiopian linseed germplasm had lower oil contents than the exotic ones. The same authors also showed that the average oil content of 13 genotypes ranged from 33 to 37%, which is closely similar to the current result (Table 6.1). However, the results of oil contents reported in India, Australia and Canada were higher than the current findings. For example, Batta *et al.* (1985) reported 37-48% in Indian germplasm accessions, while Green and Marshall (1981) demonstrated 34-46% in Australian linseed collections. Similarly, Rowland *et al.* (1988) reported 34-41% oil in some Canadian cultivars. This suggests that oil content could be further improved in Ethiopian linseed by incorporating exotic introductions into the local ones. Kenaschuk (1975) and Salas and Friedt (1995) indicated that oil content is inherited as a quantitative character and thus the prevailing growing environments could influence it besides genetic constitution of the accessions.

The second breeding objective of improving linseed oil quality can be achieved by modifying the fatty acid composition of its oil. This is possible by increasing one fatty acid at the expense of the other, as most of them have strong negative associations (Green and Marshall, 1981; Batta *et al.*, 1985). For instance, the consistent occurrence of a strong negative association between oleic acid and linolenic acid was implicated by the production of linolenic acid through the sequential desaturation of oleic acid via linoleic acid through the action of substrate-specific desaturase enzyme (Green and

Marshall, 1981; Nichterlein *et al.*, 1988). Conversely, a reduction in linolenic acid could result in an increase in the immediate precursor, linoleic acid and then in oleic acid. Hence, the significant correlations between fatty acids demonstrate the probable correlated responses that would emerge when selection was done for a particular fatty acid.

Saturated fatty acids (palmitic and stearic) were not significantly different among the accessions and they ranged between 8-12% against 85-91% of the unsaturated fatty acids (Table 6.3 and 6.7). These lower levels of saturated oils are in high demand by the health-conscious society of today. Fats and oils have acquired a poor reputation in recent years, as too much consumption of saturated fats raise blood cholesterol levels and increase the risk of heart disease (Cunnane and Thompson, 1995; Flax Council of Canada, 2000a). In this regard, linseed oil has a unique reputation due to its high linolenic fatty acid, an omega-3 fatty acid that has been shown to possess beneficial effects on heart health and autoimmune system (Cunnane *et al.*, 1995). The special feature of linseed oil lies in its the high ratio of omega-3 to omega-6 fatty acids (about 1:3.8), which is required for considerable health benefits.

Nutritionists consider these two fatty acids as essential nutrients since our bodies cannot synthesize them from any other substances; thus, we have to eat them as part of the diet (Flax Council of Canada, 2000b). Essential fatty acids (linoleic and linolenic) are required for maintaining the structure of cell membranes and permeability of the skin. Omega-3 has especially been shown to regulate gene transcription and expression, thus altering enzyme synthesis and to modify several risk factors for coronary heart disease (Cunnane *et al.*, 1995; Flax Council of Canada, 2000a). Indeed, linseed is the only plant species that contains more than half of its fat with essential omega-3 that is needed for proper growth and development of infants (Cunnane *et al.*, 1995). Research of these authors suggests that it offers protective effects against coronary heart disease, stroke, hypertension and inflammatory and autoimmune disorders. It also fights against breast, colon and other cancers (Reinhardt-Martin,

2001). Consequently, consumers are turning towards food products of linseed for their numerous health benefits and their pleasant and nutty flavours.

The amounts of palmitic and stearic fatty acids assessed in the current study (Table 6.3) were within the ranges reported by Batta *et al.* (1985) in India and by Green and Marshall (1981) in Australia. The same was also true with the level of oleic acid (monounsaturated fatty acid) that revealed significant ranges of about 14-29% among the accessions (Table 6.3). Oleic acid is important for cooking and salad oils (Green and Marshall, 1981), whereas a high level of palmitic acid is required for the production of margarine, shortening and other fat products (Ntiamoach *et al.*, 1995). The latter authors also indicated that palmitic acid is inherited as a result of the pleiotropic effect of a single additive gene. Similarly, Green (1986b) demonstrated that two independent recessive genes possessing additive gene actions control linolenic fatty acid in linseed.

The percentages of linoleic and linolenic (polyunsaturated) fatty acids in the current study were within broad ranges of 10.88-16.14 and 47.00-59.06, respectively (Table 6.3). Green and Marshall (1981) also reported similar result of 10.40-20.90% and 45.50-63.10% for linoleic and linolenic fatty acids, respectively for a diverse collection of 214 linseed accessions in Australia. Diederichsen (2001) also reported a wide range of diversity for polyunsaturated fatty acids. A strong inverse relationship was found (Green, 1986b) between the two polyunsaturated fatty acids, indicating the synthesis of linolenic from linoleic fatty acid through a desaturation process. The inverse relationship between oleic and linolenic was also reported (Batta *et al.*, 1985; Green, 1986b), suggesting the biosynthesis of oleic, linoleic and linolenic fatty acids in this order.

Accession 10256 can be used to develop cultivars rich in linolenic in contrast to accessions 10068 and 10037 that could be used to develop cultivars with good keeping quality for cooking purposes after drastically reducing their linolenic acids. Likewise, Accession N1266 had the lowest stearic and thus lowest saturated fatty acid but it had the highest unsaturated (90.58%) fatty acid. Thus, it is required for developing

cultivars rich in omega-3 fatty acid, which was much appreciated (Payne, 2000; Reinhardt-Martin, 2001) for its considerable health benefits. Accession M20G, which was grouped in cluster X had the highest linoleic acid (16.14%) together with the genotypes in cluster I. These genotypes might be useful to develop cultivars with good quality for cooking oils (Green, 1986b; Rowland *et al.*, 1995). In general, such a clustering of germplasm collections based on their mean performance and characters were also found (Greene and Pederson, 1996) useful for effective and efficient management of genetic resources without duplications of valuable resources and efforts. Friedt *et al.*, (1995) also reported the possibility of generating double-haploid lines of linseed, with remarkable variations of oil contents and fatty acid compositions. Their result shows that anther culture or haploid technique could be a useful tool for developing cultivars of linseed with different profiles of oil contents and fatty acids within few years time.

6.6 Conclusions

The present study revealed significantly useful variability in oil content, oil yield, and fatty acid profiles, indicating the possibility of improving genotypes for these characters. These high genetic variations can also result in sustainable yields by reducing the risks of crop failures, buffering various stresses. The high levels of unsaturated fatty acids and low levels of saturated ones displayed the fitness of the accessions for healthy food products enriched with omega-3 fatty acids. However, it is unlikely to obtain the required quality for cooking oil from the current variation via the conventional hybridisation techniques. Thus, more efforts are needed to acquire it through other alternatives, such as mutation breeding methods and by introducing the already developed ones.

CHAPTER 7

ASSOCIATION OF LINSEED CHARACTERS AND ITS VARIABILITY IN DIFFERENT ENVIRONMENTS

7.1 Abstract

Associations of characters were studied in linseed using data of 60 accessions evaluated in a randomised complete block design with three replications under glasshouse and field conditions in 2000 and 2001. The main objectives were to determine the magnitude of correlations between yield and its components under variable environments, and identify stable and major yield attributes that could support further improvements of linseed productivity. The degree of character association varied considerably across years and locations, due mainly to climatic factors (e.g., temperature, moisture levels, etc.) and disease incidences. However, seed yield (g/m^2) was significantly ($P < 0.01$) and positively associated with seed yield/plant, 1000-seed weight and bolls/plant across environments. These three yield attributes were also strongly and positively correlated with plant height, branches/plant, days to flowering and maturity. Oil yield was significantly and positively associated with polyunsaturated (linoleic and linolenic) fatty acids, whereas it was negatively correlated with saturated (palmitic and stearic) fatty acids. Oil yield also had a weak positive relation with monounsaturated oleic acid. The quality of linseed oil, which is dependent on the levels of these fatty acids, can be influenced by their variable correlated responses to different selection practices, besides the environmental factors. Thus, the causes and effects of these correlated responses should be well known to undertake sound and effective selection programs.

7.2 Introduction

Linseed (*Linum usitatissimum* L.) is largely a crop of temperate climates, including cooler parts of tropics, like the highlands of Africa. It is the second most important oil crop in the highlands of Ethiopia (Adefris *et al.*, 1992), where small-scale farmers have been producing it organically, without applying any chemicals (fertilizers, herbicides, etc.) and with minimum inputs (Adugna, 2000). It has been

grown in Ethiopia primarily for food and to generate cash revenues for the farmers either on local markets or by exporting abroad. For food, the seeds are usually roasted, ground and mixed with spices and some water to be served along with cooked and boiled food products (Adugna, 2000). Limited amounts of linseed is also locally pressed for its edible oil that is often blended with oils of other oil crops that have high oil quality, such as niger seed (*Guizotia abyssinica* CASS) that has about 75% linoleic acid (Getinet and Adefris, 1992).

Recent reports (Carter, 1993; Cunnane *et al.* 1995; Payne, 2000; Reinhardt-Martin, 2001) indicate that consumption of linseed imparts numerous health benefits, including the anti-hypercholesterolemic, anti-carcinogenic and essentiality in the developments of brain and retinal tissues of infants. In order to maximize such benefits, one has to increase the productivity of the crop directly or indirectly via its yield components. According to Lafond (2001), the three major yield components in linseed are number of bolls per unit area, number of seeds/boll and seed weight. Moreover, recent studies (Hassan *et al.*, 1999; Lafond, 2001; Adugna and Labuschagne, 2002) have shown years, locations, seeding date, nitrogen fertility, variety and seeding rates were the factors that have the greatest influence on final seed yield of linseed, provided proper weed control, crop establishment and fertility conditions were fulfilled. Besides, correlations of characters with yield are useful in linseed breeding to provide the criteria for indirect selection of the characters (Mishra and Maheshwari, 1975). Such studies can provide reliable information on the nature, extent and direction of selection. Thus, knowledge of interrelationships between different traits is important in breeding for direct and indirect selection of characters that are not easily measured and those with low heritability (Patil *et al.*, 1981).

Several studies (Mishra and Maheshwari, 1975; Agrawal, *et al.*, 1994; Kurt, 1996) were carried out on correlations of characters in linseed. Mahto and Mahto (1998) studied the variability and correlation for seven yield-related characters of linseed genotypes and found that seed yield/plant, bolls/plant, secondary branches and primary branches/plant had high correlations with yield. They also reported that seed yield/plant was correlated with days to maturity, plant height, primary branches/plant, secondary branches/plant and bolls/plant at the genotypic level.

Moreover, Kurt (1996) showed significant positive correlations between seed yield/plant and bolls/plant, and between height of first branch and bolls/plant and seed yield/plant. The same author also reported significant negative correlations between 1000-seed weight and height of first upper branch, between bolls/plant and seeds/boll, and between seed yield/plant and 1000-seed weight.

Similar correlation analysis of 72 eco-geographically divergent varieties of linseed indicated that seed yield had a high positive correlation with bolls/plant and moderate positive correlation with seeds/boll, and significant negative correlation with 1000-seed weight (Muduli and Patnaik, 1994). According to these workers, bolls/plant and seeds/boll were the major components determining yield. Rashid *et al.* (1998) also indicated that seed yield (g/m^2) had a positive and significant correlation with bolls/plant, seeds/boll, 1000-seed weight and oil content, whereas days to flowering and plant height had negative correlations with seed yield (g/m^2). They also indicated that protein content and oleic acid had a negative association with seed yield (g/m^2). Furthermore, they suggested that bolls/plant and oil content could be a reliable selection index for maximum oil yield per unit area.

Nevertheless, data on correlation studies showing the influence of environments in the expression of correlated characters are generally lacking in linseed. But yield is a complex character determined by several components. It is also quantitatively inherited and influenced by genotype, environment and their interactions (GEI). Hence, understanding of such important association of characters is necessary to conduct effective selection programs. The identification of yield contributing plant characters is also important as they improve breeding efficiency. GEI results because individual genotypes differ in their responses to variations in soil fertility, soil moisture, temperature, day-length, light intensity, humidity, disease, cultural practices, or other environmental factors (Poehlman, 1987; Basford and Cooper, 1998). GEI reduces association between phenotypic and genotypic values (Romagosa and Fox, 1993), and may cause selections from one environment to perform poorly in another. Measurement of GEI is also important to determine an optimum breeding strategy for releasing genotypes with adequate adaptation to their target environments. Such a study of GEI is especially relevant for countries, like Ethiopia that has very diversified agro-ecologies (MoA, 1998). Thus, an

attempt was made here to study the role of different testing environments in character association of diverse accessions of linseed. Its specific objective was to examine the magnitude of correlations between different characters of linseed under glasshouse and field conditions in order to identify major characters for achieving higher yield. The premise is that, by evaluating broad genetic materials of linseed in diverse environments, more reliable information could be obtained to contribute to the future breeding and development efforts in a sustainable manner.

7.3 Materials and methods

Plant materials

The accessions used in this study were selectively taken from the Highland Oil Crops Research Program of Holeta Research Centre in the Ethiopian Agricultural Research Organization to represent the apparent diversity of linseed germplasm in the country. Totally 60 accessions, including four released varieties (Chilalo, CI-1525, CI-1652 and Belay 96) were studied for their character association and variability across environments. Belay 96 was developed and released in 1996 out of a crossing program undertaken between locally well-adapted and disease resistant collections at Holeta Research Centre. Fifty-three accessions were collected from different regions of Ethiopia and Eritrea (Fig. 3.1), the remaining seven being introductions from Europe, USA and Canada (Table 3.1).

Experimental environment and methods

Glasshouse

The study at Bloemfontein was conducted in two different glasshouses during 2000 and 2001 at the University of the Free State (latitude 29° 6' S, longitude 26° 18' E, altitude 1351 m above sea level) in South Africa. The first glasshouse used in 2000 was warmer by about 10°C (average) than the one used in 2001. The latter had a maximum temperature of up to 25°C during the daytime and the minimum night temperatures ranged between 8 and 13°C in both glasshouses. Daylight hours ranged from 10.1 in June to 13.9 in December, and April to September had relatively shorter, 11.3-11.8 hours of daylight. The accessions were evaluated in pots (2.5 litres) that were laid out in a randomised complete block design with three replications. Eight seeds were sown at a depth of about 2.5 cm and thinned to

five plants per pot three weeks after planting. The seeds were sown in early September during the first year and in mid June during the second year. A mixture of nitrogen and phosphorus fertilizers of 3 g per pot was applied to red sandy-loam soil (pH = 5.8 in water) at sowing, 50% flowering and seed filling stages. The plants were irrigated almost every day from planting to maturity.

Field experiment

The field trial was conducted at Holeta Research Centre (latitude 9° 3' N, longitude 38° 30' E, altitude 2400 m above sea level) during the 2000 and 2001 main cropping seasons in Ethiopia. Monthly temperature ranged between 2-22°C during the growing seasons (June-November), while total rainfall varied between 900-1000 mm per annum during the two growing years. The minimum temperature, 2°C occurred during nights in November after the crop was already matured. The accessions were evaluated in two rows (5 m long spaced at 20 cm = 2 m^2) that were laid out in a randomised complete block design with three replications. The seeds were planted at a seeding depth of about 2.5 cm on June 26 during both years. The recommended fertilizers of both nitrogen (N) in the form of urea and phosphorus (P_2O_5) in the form of diammonium phosphate (DAP) were manually drilled and incorporated with the red clay (eutric Nitosol) soil both at a rate of 23 kg/ha at planting. The seeds were also drilled by hand at a rate of 25 kg/ha. Weeds were controlled by hand weeding and no herbicide or insecticide was applied. The plants of each accession was separately harvested and dried for about 15 to 30 days, threshed and cleaned manually. Seed yield data were taken at about 8% seed moisture level. Seeds were oven dried to about 1% seed moisture level for oil analysis.

Data recording

Data on the agronomical and morphological characters were collected from three and five randomly selected plants in the glasshouses and in the field, respectively and their means were recoded for all observations. Except for days to flower initiation, 50% flowering, diseases scores and similar observations, most of the data were recorded at maturity and thereafter. Scores for powdery mildew (*Odium* spp.) and pasmo (*Septoria linicola*) diseases were recorded on 0-5 scales (0 = nil; 5

= very severe) under normal field conditions without any artificial inoculation. Days to flowering and maturity represent the number of days from sowing to 50% blooming and physiological ripening, respectively. The unit of measurement for plant height was centimetres (cm), while that of seed yield and 1000-seed weight was in grams (g). Oil yield (g/m^2) was estimated by multiplying the mean values of oil content (%) and seed yield (g/m^2) for each accession. In general, most of the evaluation was undertaken based on the major phenological and morphological quantitative descriptors (Diederichsen, 2001) that are reported to display high heritability values.

Analyses of oil content and fatty acids

Oil contents were determined by a wide line nuclear magnetic resonance (NMR) and solvent extraction method (SEM). About 25 g of oven-dried seeds were analysed by NMR (Newport Analyser) with reference to a standard of extracted linseed oil at Holeta Research Centre in Ethiopia. In South Africa, however, oils were extracted (Appendix 3) for fatty acid analysis from about 0.5 g of ground seed of linseed, following the organic solvent extraction method (Folch *et al.*, 1957) in the laboratories of Food Science at the University of the Free State. Fatty acid methyl esters were prepared following the method of Slover and Lanza (1979). Fatty acids were quantified using a Varian GX 3400 flame ionisation gas chromatograph, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 μm ID, 0.2 μm film thickness). Column temperature was 40-230°C (hold 2 minutes; 4°C/min.; hold 10 minutes).

Fatty acid methyl esters in hexane (1 μl) were injected into the column using a Varian 8200 CX Autosampler with a split ratio of 100:1. The injection port and detector were both maintained at 250°C. Hydrogen was used as the carrier gas at 45 psi and nitrogen was the makeup gas. Chromatograms were recorded with Varian Star Chromatography Software. Identification of sample fatty acids was made by comparing the relative retention times of fatty acid methyl ester peaks from samples with those of standards obtained from SIGMA (Cat. No. 189-19).

Statistical analysis

The mean values of all observations for the measured characters were first screened for normality and outliers by using the Number Cruncher Statistical System, NCSS 2000 (Hintze, 1998) statistical system for Windows. Then, data that showed non-normal distribution were transformed by a square root system and analysed for their correlations and significance using the same statistical software. The total observations for each location and year were separately analysed first and then, their combined data were analysed to investigate their correlations across environments, locations and years. The agronomic characters were also analysed for their combined variance and significance using AGROBASE 98 software (Agronomix Software, Inc., 1998).

7.4 Results

Separate analyses

Glasshouse experiment

The analyses of correlations between the evaluated characters for the separate years of 2000 and 2001 were presented in Table 7.1. Seed yield (g/m^2) was significantly ($P < 0.01$) and positively correlated with plant height and seeds/boll in 2000, while it was strongly associated with only seed yield/plant ($r = 0.8998$) in 2001. Likewise, seed yield/plant was significantly and positively correlated with plant height, number of primary and secondary branches/plant in 2000 but this performance was not repeated in the succeeding year, showing fluctuations in character associations due to environmental variables, specifically because of differences in temperature of the two glasshouses used, as already described in the materials and methods section.

Seed yield/plant was negatively correlated with days to flowering and maturity during both years, indicating the low yielding traits of early maturing genotypes. On the other hand, bolls/plant was strongly related with primary and secondary branches in both years, and it was also significantly associated with plant height in 2001. In the same manner, 1000-seed weight was strongly associated with yield/plant, seed yield (g/m^2), plant height and bolls/plant in 2000 and it was also significantly correlated with the former two traits in 2001.

Field experiment

Under the Holeta field conditions of Ethiopia, seed yield (g/m^2) was significantly ($P < 0.01$) and positively correlated with plant height, days to flowering, days to maturity, seed yield/plant and 1000-seed weight in 2000 and the first three characters repeated their performance in the year 2001 too (Table 7.2). Moreover, primary branches/plant and bolls/plant significantly contributed to seed yield (g/m^2) in 2001 in contrast to their non-significant effects in the previous year.

The incidence of powdery mildew negatively affected the yield, unlike pasmo score that had non-significant effects on the seed yield. Both diseases had a significant ($P < 0.01$) negative correlation with plant height, days to flowering and maturity, suggesting that shorter and early maturing genotypes were susceptible to these diseases. The two diseases also showed significant positive association with each other, indicating similar reactions of the genotypes to these diseases, and this result was in close agreement with a recent study (Adugna, 2000).

The other yield component of linseed, bolls/plant was significantly and positively associated with plant height, primary branches and secondary branches during both years. Likewise, 1000-seed weight was positively correlated with plant height, but it was negatively associated with days to flowering, powdery mildew and seeds/boll during both years. Nevertheless, the relationship of 1000-seed weight with seed yield was highly significant ($P < 0.05$), and this confirmed its positive contribution to the seed yield.

Similarly, plant height was consistently and strongly correlated with days to maturity, bolls/plant, yield/plant, seed yield (g/m^2) and 1000-seed weight, reflecting the important contribution of taller genotypes to seed yield and its components. Primary branches were positively associated with yield/plant, and secondary branches and plant height were strongly correlated with bolls/plant during both years. Mahto and Mahto (1998) also found that seed yield/plant was positively correlated with plant height, primary branches, secondary branches and bolls/plant.

Table 7.1. Separate analysis of correlation coefficients between 11 quantitative traits of 60 linseed accessions evaluated in 2000 (bold and upper diagonal) and in 2001 (lower diagonal) at UFS, South Africa

Trait	PH	IDF	DF	DM	PB/P	SB/P	B/P	S/B	SY/P	TSY	TSW
PH		**0.3992****	**0.3464****	**0.1893***	**0.0991**	**0.1819***	**0.1247**	**-0.0152**	**0.2664****	**0.2430****	**0.2043****
IDF	0.2090**		**0.8530****	**0.5266****	**-0.0066**	**0.0338**	**0.0275**	**0.0581**	**0.0099**	**-0.1596***	**-0.2637****
DF	0.2277**	0.7960**		**0.5645****	**-0.0703**	**0.0007**	**-0.0156**	**0.0435**	**-0.0067**	**-0.2312****	**-0.3662****
DM	0.1070	0.3327**	0.4471**		**-0.0444**	**0.0457**	**0.1411**	**-0.1160**	**0.1721***	**-0.4262****	**-0.1430**
PB/P	0.0713	0.1249	0.0784	-0.0724		**0.3927****	**0.4430****	**0.0079**	**0.2913****	**0.0794**	**0.1355**
SB/P	0.3663**	-0.0529	0.0107	-0.0257	0.3034**		**0.6709****	**-0.1435**	**0.4895****	**0.0412**	**0.0321**
B/P	0.2350**	-0.0498	-0.0004	-0.0487	0.3316**	0.5127**		**-0.1199**	**0.6076****	**0.0722**	**0.1488***
S/B	-0.0639	0.1180	0.1356	0.0055**	-0.0045	-0.0968	0.0092		**-0.2725****	**0.3612****	**-0.2196****
SY/P	0.0608	-0.0769	-0.1838*	-0.4262**	0.0650	0.0737	0.1034	-0.0279		**0.0793**	**0.2542****
TSY	0.0597	-0.0793	-0.1866*	-0.4255**	0.0657	0.0720	0.1040	-0.0296	0.8998**		**0.3612****
TSW	-0.1822	0.0809	-0.1130	-0.0919	-0.0359	-0.4200**	-0.2764**	-0.1125	0.1476*	0.1476*	

*, ** = Significant at $P < 0.05$, 0.01, respectively; PH = Plant height at maturity; IDF = Initial days of flowering; DF = Days to 50% flowering; DM = Days to maturity; PB/P = Primary branches/plant; SB/P = Secondary branches/plant; B/P = Bolls/plant; S/B = Seeds/boll; SY/P = Seed yield/plant; TSY = Total seed yield (g/m^2); TSW = Thousand seed weight; N = 180

Table 7.2. Separate analysis of correlation coefficients between 11 quantitative traits of 60 linseed accessions evaluated in 2000 (bold and upper diagonal) and 2001 (lower diagonal) at HRC, Ethiopia

Trait	PH	DF	DM	PM	PS	PB/P	SB/P	B/P	S/B	SY/P	TSY	TSW
PH		**0.0501**	**0.5127****	**-0.1469***	**0.0165**	**0.1132**	**-0.0511**	**0.2086****	**0.1073**	**0.4635****	**0.6222****	**0.1868****
DF	0.0.0168		**01467***	**0.0078**	**-0.2469****	**0.0817**	**-0.0172**	**-0.1520***	**0.2027****	**-0.0808**	**0.1680***	**-0.1097**
DM	0.3817**	0.4051**		**-0.0250**	**-0.1903***	**-0.0037**	**0.0222**	**0.0140**	**0.0523**	**0.1455***	**0.2773****	**0.0138**
PM	-0.5245**	-0.1990**	-0.4086**		**-0.0129**	**-0.1506***	**0.0226**	**-0.0382**	**-0.0547**	**-0.0936**	**-0.0479**	**-0.0989**
PS	-0.1144	-0.3430**	-0.2195**	0.2206**		**-0.0353**	**-0.2173****	**0.0507**	**-0.0700**	**0.0286**	**0.1198**	**0.1210**
PB/P	0.1477*	0.1810*	0.1081	-0.1156	0.0585		**0.5318****	**0.3807****	**0.1687****	**0.2114****	**0.0679**	**0.0979**
SB/P	-0.1472*	0.0093	-0.1054	0.2154**	-0.0629	0.4381**		**0.4177****	**0.0931**	**0.1523***	**-0.0607**	**0.0110**
B/P	0.2535**	-0.1396	0.0151	-0.1327	0.1447	0.4015**	0.2952**		**0.0182**	**0.6918****	**0.0764**	**0.0531**
S/B	-0.0277**	0.0384	0.0015	0.0495	-0.1125	-0.0743	-0.2099**	-0.2058**		**0.1433**	**0.0798**	**-0.0739**
SY/P	0.3269**	-0.2037**	-0.1657*	0.0043	-0.0235	0.0687	0.0500	0.4827**	0.3153		**0.2232****	**0.1698***
TSY	0.4186**	0.0286	0.2358**	-0.4114**	0.1072	0.2438**	-0.0686	0.2703**	0.0082	0.0301		**0.1458***
TSW	0.5568**	-0.2905**	0.0173	-0.2532**	0.0890	-0.0947	-0.1152	0.1600*	-0.1477*	0.4547**	0.1255	

*, ** = Significant at P < 0.05, 0.01, respectively; PH = Plant height at maturity; IDF = Initial days of flowering; DF = Days to 50% flowering; DM = Days to maturity; PB/P = Primary branches/plant; SB/P = Secondary branches/plant; B/P = Bolls/plant; S/B = Seeds/boll; SY/P = Seed yield/plant; TSY = Total seed yield (g/m^2); TSW = Thousand seed weight; N = 180.

Combined analyses

Across locations

The combined data analysis across locations revealed that seed yield (g/m^2) was significantly ($P < 0.01$) and positively correlated with seed yield/plant and 1000-seed weight under glasshouse conditions. It was also strongly associated with plant height, days to flowering and maturity, primary branches/plant, bolls/plant and 1000-seed weight under field conditions (Table 7.3). That means, the association between seed yield (g/m^2) and 1000-seed weight was significantly correlated under both glasshouse and field environment. Similar results were also reported by other researchers (Mishra and Maheshwari, 1975; Rashid *et al.*, 1998).

On the other hand, seed yield (g/m^2) was significantly ($P < 0.01$) and negatively correlated with powdery mildew under field conditions. In fact, both powdery mildew and pasmo negatively affected the seed yield and its yield components (Table 7.3) at Holetta in Ethiopia, where these diseases often occur. In contrast, seed yield/plant was positively correlated with plant height, days to flowering and maturity, primary branches, secondary branches and bolls/plant in the glasshouses, and it had the same relationship with plant height, primary branches, secondary branches, bolls and seeds/boll under field conditions. In other words, plant height, primary branches/plant, secondary branches/plant and bolls/plant were significantly correlated with yield/plant under both environments.

Mishra and Yadav (1998) also revealed a significant positive correlation between seed yield and bolls/plant, seeds/boll and branches/plant. Similarly, bolls/plant were significantly ($P < 0.01$) correlated with plant height, days to flowering and maturity, and primary and secondary branches in the glasshouses. It was also strongly associated with plant height, primary and secondary branches under field conditions. That means, plant height, primary and secondary branches had a strong positive association with bolls/plant, while the remaining relationships were varied under both glasshouse and field environments. This variability was also confirmed by the occurrence of significant interactions of characters with their environments (Table 7.6). In this regard, the minimum, maximum and mean values of the studied characters (Table 7.7) showed a broad spectrum of variation. Almost all of these traits showed significant ($P < 0.05$) differences among accessions, and more than

double values of ranges were observed between their minimum and maximum values. All of these evidences further denote the presence of abundant genetic diversity among the evaluated accessions of linseed, and these results are also an indicative of that Ethiopia is the centre diversity for linseed (Adefris *et al.*, 1992; Adugna, 2000).

Table 7.3. Correlation coefficients between the combined data of different traits of 60 linseed accessions evaluated at UFS (bold and upper diagonal) and HRS (lower diagonal), 2000-2001

Trait	PH	DF	DM	PM	PS	PB/P	SB/P	B/P	S/B	SY/P	TSY	TSW
PH		**0.7734****	**0.7424****	-	-	**0.1858****	**0.3347****	**0.3565****	**-0.0094**	**0.7010****	**0.0660**	**0.3616****
DF	0.0642		**0.8766****	-	-	**0.1719****	**0.1808****	**0.2597****	**0.0632**	**0.7144****	**-0.0925**	**0.4209****
DM	0.5667**	0.2746**		-	-	**0.1319****	**0.1846****	**0.2768****	-0.0077	**0.7055****	**-0.1851****	**0.4509****
PM	-0.3946**	-0.1230*	-0.3195**		-	-	-	-	-	-	-	-
PS	0.0419	-0.2789**	-0.0985	0.0837		-	-	-	-	-	-	-
PB/P	0.1810**	0.1374**	0.1117*	-0.1601**	0.0409		**0.3480****	**0.3904****	**0.0041**	**0.2040****	**0.0684**	**0.0866**
SB/P	-0.1381**	-0.0132	-0.0917	0.1579**	-0.1520**	0.4180**		**0.5810****	**-0.1131****	**0.2600****	**0.0580**	**0.1261****
B/P	0.2631**	-0.1339*	0.0765	-0.1151*	0.1173*	0.4014**	0.3389**		**-0.0319****	**0.3411****	**0.0898**	**-0.0025**
S/B	-0.0249	0.0968	-0.0359	0.0435	-0.1164*	0.0130	-0.0624	-0.1105*		**-0.0291**	**0.0368**	**-0.0889**
SY/P	0.2556**	-0.1529**	-0.0996	0.0152	-0.0365	0.1074*	0.1192*	0.5477**	0.2592**		**0.4058****	**0.5286****
TSY	0.6649**	0.1364**	0.4389**	-0.2770**	0.1058	0.1815**	-0.1195*	0.1840**	-0.0323	0.0086		**0.1495****
TSW	0.3070**	-0.1983**	0.0139	-0.1804**	0.1012	0.0059	-0.0506	0.0984	-0.1144*	0.3068**	0.1093*	

*, ** = Significant at $P < 0.05$, 0.01, respectively; PH = Plant height at maturity; DF = Days to 50% flowering; DM = Days to maturity; PM = Powdery mildew score; PS = Pasmo score; - = not available; PB/P = Primary branches/plant; SB/P = Secondary branches/plant; B/P = Bolls/plant; S/B = Seeds/boll; SY/P = Seed yield/plant; TSY = Total seed yield (g/m^2); TSW = Thousand seed weight; N = 360

Table 7.4. Correlation coefficients between the combined data of different traits of 60 linseed accessions evaluated in four environments of UFS and HRC, 2000-2001

Trait	PH	DF	DM	PB/P	SB/P	B/P	S/B	SY/P	TSY
DF	0.7709**								
DM	0.7397**	0.8930**							
PB/P	0.1854**	0.1719**	0.1319**						
SB/P	0.3341**	0.1808**	0.1846**	0.3480**					
B/P	0.3534**	0.2590**	0.2762**	0.3913**	0.5822**				
S/B	-0.0091	0.0632	-0.0077	0.0041	-0.1131**	-0.0322			
SY/P	0.4009**	0.3530**	0.3168**	0.1328**	0.1567**	0.2174**	-0.0020		
TSY	0.0700	-0.0902*	-0.1835**	0.0695	0.0552	0.0888*	0.0387	0.5512**	
TSW	0.3691**	0.4210**	0.4507**	0.0867*	-0.1264*	-0.0035	-0.0884*	0.3342**	0.1515**

*, ** = Significant at P < 0.05, 0.01, respectively; PH = Plant height at maturity; DF = Days to 50% flowering; DM = Days to maturity; PB/P = Primary branches/plant; SB/P = Secondary branches/plant; B/P = Bolls/plant; S/B = Seeds/Boll; SY/P = Seed yield/plant; TSY = Total seed yield (g/m^2); TSW = Thousand seed weight; N = 720.

Likewise, 1000-seed weight had a strong and positive correlation with days to flowering, days to maturity, primary branches/plant, plant height, yield/plant and seed yield (g/m^2) in glasshouses and the three latter characters repeated the same performance under field conditions (Table 7.3). However, 1000-seed weight was negatively correlated with days to flowering, powdery mildew and seeds/boll under the field environment. A similar significant negative association was also reported (Satapathi *et al.*, 1987) between days to maturity and 1000-seed weight.

Across locations and years

The combined data analysis across sites and years revealed that seed yield (g/m^2) was positively correlated with seed yield/plant, 1000-seed weight and bolls/plant, while it was negatively correlated with days to flowering and maturity, indicating that early maturing genotypes were relatively poor yielding (Table 7.4). Seed yield/plant was also significantly ($P < 0.05$) and positively correlated with plant height, days to flowering and maturity, primary and secondary branches and bolls/plant. Similarly, 1000-seed weight was strongly associated with plant height, days to flowering and maturity, primary branches and with seed yield; however, it was negatively related with secondary branches, bolls/plant and seeds/boll.

Oil yield and fatty acid composition

Oil yield (g/m^2), which is the product of seed yield and oil content, was significantly ($P < 0.01$) and positively correlated with polyunsaturated (linoleic and linolenic) fatty acids, while negatively associated with saturated (palmitic and stearic) fatty acids (Table 7.5). Oil yield also showed a weak correlation with monounsaturated (oleic) fatty acid. Mishra and Maheshwari (1975) reported that seed yield of linseed was positively correlated with oil content, seeds/boll and 1000-seed weight, indicating that these characters significantly contribute to the oil yield in linseed. Since the association between seed yield and oil content was positive, the improvement for seed yield or oil content could enhance the other character and simultaneous improvement of both traits would be possible.

The mean of oil content was only positively related with the total unsaturated fatty acids, and with both solvent extraction method and nuclear magnetic resonance (SEM and NMR), indicating both oil extraction methods were good enough to measure the

oil contents of linseed. However, the correlation coefficient of SEM (r = 0.8934) was relatively higher than that of NMR (r = 0.6949), showing the former technique was better than the latter in quantifying the percentage of linseed oil. Oil yield was also significantly ($P < 0.01$) and positively associated with both methods of oil extraction (Table 7.5).

There was a strong negative association between the total saturated fatty acids and the unsaturated fatty acids, indicating the desaturating processes from palmitic to linolenic fatty acids during their biosynthesis (i.e., palmitic → stearic → oleic → linoleic → linolenic) (Batta *et al.*, 1985; Green, 1986b; Nigussie, 2001). Linolenic fatty acid was strongly correlated (r = 0.6534) with linoleic acid. This result did not agree with that of Green (1986b), which indicated a complete inverse correlation between linoleic and linolenic. Batta *et al.* (1985) also reported a weak correlation between linoleic and linolenic fatty acids. This shows that correlation results can be variable depending on genotypes, environments, G x E interactions and other factors, such as extraction processes (i.e., methods, timing, storage conditions, etc.). Therefore, it is very important to take into consideration all the factors that could affect the oil yield and fatty acid conditions, including their testing environments to obtain more reliable results.

Table 7.5. Correlation coefficients (r) between oil contents and fatty acids of 60 linseed accessions analysed UFS, 2001

Trait	Palmitic (C16:0)	Stearic (C18:0)	Total saturated	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)	Total un-saturated	Oil % (SEM)	Oil % (NMR)	Oil % (Mean)
C18:0	0.3505**									
TS	0.7219**	0.9011**								
C18:1	0.2276*	0.2929*	0.3218*							
C18:2	-0.4611**	-0.4258**	-0.5281**	-0.7637**						
C18:3	-0.3092*	-0.4078**	-0.4445**	-0.9610**	0.6534**					
TU	-0.7615**	-0.7909**	-0.9369**	-0.2611*	0.4733**	0.4118**				
SEM	-0.4154**	0.0403	0.1626	0.2162*	-0.0364	-0.1867	0.1897			
NMR	-0.3280*	-0.3478**	-0.4088**	0.0408	0.3549**	-0.0728	0.3873**	0.2977*		
Oil % (M)	-0.4675**	-0.1335	-0.3150*	0.1822	0.1398	-0.1751	0.3254*	0.8934**	0.6949**	
Oil yield	-0.3028*	-0.3413**	-0.3924**	0.0067	0.3094*	0.8586**	0.3587**	0.5537**	0.6584**	0.7271**

*, ** = Significant at $P < 0.05$, 0.01, respectively; TS = Total saturated fatty acids; TU = Total unsaturated fatty acids; Oil contents determined by SEM = Solvent extraction method, and NMR =Nuclear magnetic resonance M = Mean of SEM and NMR; N = 60

Table 7.6. Mean squares of the combined analysis of variance for the major characters of 60 linseed accessions (entries) across four environments (UFS and HRC, 2000-2002)

Source	df	Traits									
		PH	DF	DM	PB/P	SB/P	B/P	S/B	SY/P	TSY	TSW
Year	1	**	**	**	*	**	*	*	**	**	**
Location (L)	1	**	**	**	*	**	**	**	NS	NS	NS
Y x L	1	**	**	**	**	NS	**	*	NS	NS	**
Entry (E)	59	**	**	**	**	**	**	**	**	**	**
E x Y	59	**	NS	**	NS	NS	NS	NS	*	**	NS
E x L	59	**	**	**	*	**	*	NS	NS	**	NS
E x Y x L	59	**	**	**	NS	NS	NS	NS	NS	NS	NS
Rep (Y x L)	8	NS	NS	**	NS	**	**	**	**	**	**
Residual	472	49.98	27.08	54.20	0.47	1.89	23.84	1.54	0.05	1079.80	1.97

*, ** = Significant at $P < 0.05$, 0.01, respectively; PH = Plant height; DF = Days to 50% flowering; DM = Days to maturity; PB/P = Primary branches/plant; SB/P = Secondary branches/plant; B/P = Bolls/plant; S/B = Seeds/boll; SY/P = Seed yield/plant; TSY = Total seed yield (g/m^2); TSW = Thousand seed weight

Table 7.7 Overall mean performances of 60 linseed accessions evaluated at UFS and HRC, 2000-2001

Character	Minimum	Maximum	Mean	CD[1] 0.05	CV
Plant height (cm)	56	88	70.08	9.53	10.09
Days to flowering	70	92	82.07	7.02	6.34
Days to maturity	135	153	144.00	9.93	5.13
Primary braches/plant	3	5	4.20	0.92	16.36
Sec. branches/plant	4	8	6.17	1.85	17.31
Bolls/plant	13	25	17.40	6.58	18.07
Seeds/boll	7	10	9.11	1.67	13.63
Seed yield/plant (g)	0.51	1.08	0.70	0.30	22.14
Total seed yield (g/m^2)	51	127	86.14	22.16	10.29
1000-seed weight (g)	5	9	8.20	1.89	12.10
Oil content (%)	29.13	35.88	32.06	0.35	5.05
Total oil yield (g/m^2)	1443	3276	2243	79.39	0.08
Palmitic acid (%)	5.19	6.57	6.03	6.33	4.87
Stearic acid (%)	2.86	5.70	4.88	10.11	9.62
Oleic acid (%)	14.76	29.33	22.56	0.74	0.15
Linoleic acid (%)	10.88	16.14	13.36	0.23	8.16
Linolenic acid (%)	47.00	59.06	51.86	0.62	5.53

[1]CD = Critical difference; CV = Coefficient of variability

7.5 Discussion

Studies of character association across environments may supply more reliable information on the nature and level of interrelationships of linseed yield with its yield components. Economic characters, such as seed and oil yields are controlled by many genes, and have a complex type of inheritance (Poehlman, 1987). Thus, they are much influenced by the environment. The progress of breeding for such economic traits is determined by the magnitude and nature of their genotypic variability. Hence, the estimates of correlations of yield with its components are very important to utilize the available variability through different selection methods. With these premises in mind, association of linseed characters and their variability in different environments was conducted to determine the degree of

correlations between yield and its components in order to detect consistent and major yield attributes on which future genetic improvement should be based.

The results of both glasshouse and field experiments indicated variable degrees of correlations among the different characters analysed. There were significant variations across the years and localities. These results were also substantiated by the combined analysis of variance (Table 7.6) that revealed highly significant differences for genotypes, years, locations and their interactions for most of the characters. This indicates the very high differential expressions of the genotypes and their quantitative traits in response to the variable environmental factors. Since the quantitative traits are greatly influenced by environmental factors and show continuous variation, their results from single environment (one year or one locality) are less reliable as compared to the multi-environment results. That is why multi-environment tests are preferred in many breeding and agronomic experiments. This is specifically true for countries like Ethiopia, which is characterized by a diversity of climate, physiography, soils, vegetation, farming systems and socio-economic conditions (Amsalu, 2001). Since quantitative characters and their associations are the function of genes, environment and their interactions, joint analysis of these factors may lead to better understanding and more reliable results. By increasing replications, testing sites and years, selection responses and gains can be increased (Atlin *et al.*, 2001). This can improve the quality of information obtained for improving effectiveness of the breeding program through more reliable yield attributes.

Temperature was reported (Kenaschuk, 1975; Green, 1986b; Adugna and Adefris, 1995; Adugna, 2000) to be the most important environmental factor influencing yield, oil content, maturity period, plant height, fatty acid levels and diseases of linseed. High temperature at flowering stage was reported (Green, 1986b) to be deleterious for boll setting, depress seeds/boll and seed weight. Luhs and Friedt (1994) indicated that higher temperatures and drought during the sensitive seed-filling periods accelerate maturity, and thus reduce seed size and oil content that normally ranges from about 32 to 45% depending on variety, seed size, climate and maturity. Green (1986b) reported the decline of oil content by 4% as temperature increased from 15/10 to 27/22 (day/night) degree centigrade. This author also

found that increased temperature increased saturated fatty acids, while decreasing the unsaturated ones, particularly linoleic and linolenic acids. A range of about 12-18°C was reported (Getinet and Nigussie, 1992) to be suitable for normal growth and development of linseed in Ethiopia and extreme temperatures outside of this range could affect the crop drastically.

The other environmental factor that greatly affects the yield of linseed was moisture stress (Kenaschuk, 1975), especially from the first two to five weeks after flowering. This author indicated that boll setting, seeds/boll and seed weight were reduced in the flowers formed latter in the season due to moisture stress. A very high flower abortion rate was also reported (Adugna, 2000) at Holetta in Ethiopia when dry spells occurred during flowering periods. Thus drought and temperature are the most important environmental factors observed to affect the yield performance of linseed by influencing the yield components and their correlations. Kenaschuk (1975) indicated that yield improvement of linseed could be achieved by selecting for individual yield components like bolls/plant, seeds/boll and 1000-seed weight. He also indicated that both additive and non-additive genetic effects were significant for yield and its components.

Plant density, 1000-seed weight, seeds/boll and bolls/plant need to be considered in cultivar improvement schemes besides tillering and lodging that are much influenced by plant density and nitrogen supply (Luhs and Friedt, 1994). These authors have estimated the yield of modern linseed cultivars can reach up to 3 ton/ha under optimum conditions though realisation of this potential is often limited by economic and ecological conditions. Patil *et al.* (1981) found that yield/plant of linseed was positively correlated with bolls/plant and seeds/bolls, whereas Kurt (1996) reported a negative association between yield/plant and 1000-seed weight, bolls/plant and seeds/boll. These observations indicate that there could not be an improvement in total seed yield simply by increasing only seeds/boll, as it reduces the seed weight and consequently the yield. So, one has to deal with the other major yield components, such as yield/plant, 1000-seed weight and bolls/plant that were significantly, positively and consistently associated with seed and oil yield.

Oil yield was significantly and positively correlated with polyunsaturated (linoleic and linolenic) fatty acids, whereas negatively associated with the saturated (palmitic and stearic) ones (Table 7.5). Similarly, Mishra and Maheshwari (1975) reported that seed yield was positively correlated with oil content, seeds/boll and 1000-seed weight, indicating that these characters significantly contribute to the oil yield in linseed. Since the association between seed yield and oil content was positive, the improvement for seed yield or oil content can enhance the other character and simultaneous improvement of both traits could be possible. Moreover, the average of oil content was positively correlated with the total unsaturated fatty acids and with both oil extraction methods, reflecting that both methods were good in quantifying the oil contents of linseed.

Linoleic was negatively associated with all the saturated fatty acids and oleic acid, and this latter monounsaturated fatty acid was positively correlated ($P < 0.05$) with saturated fatty acids (Table 7.5). The saturated fatty acid was observed to be associated positively only with its close components, particularly with stearic acid. Since the quality of linseed oil is dependent on its fatty acid composition and they are influenced by various factors, e.g., genotypes, environments and correlated responses of fatty acids, all of these components should be considered to achieve the targeted oil quality. Moreover, since yield of linseed is dependent on a number of related traits, understanding of such interrelationship of characters under different environments was found essential in formulating selection criteria and in enhancing breeding progress of linseed.

7.6 Conclusions

The magnitude of character associations showed a wide range of variation across years and locations, due mainly to environmental factors, such as temperature levels, moisture regimes and disease incidences. However, seed yield of linseed per unit area was significantly and positively associated with seed yield/plant, 1000-seed weight and bolls/plant across environments. These three yield attributes were also strongly and positively correlated with plant height, branches/plant, days to flowering and maturity. These results show that association of characters among components of economic worth is of considerable importance in improving the efficiency of linseed yield via different selection programs.

CHAPTER 8

DIVERSITY ANALYSIS OF LINSEED USING AFLP MARKERS

8.1 Abstract

Linseed (*Linum usitatissimum* L.) is the second most important oil crop in the highlands of Ethiopia where it has been cultivated for its valuable seed-oil since antiquity. Sixty accessions of linseed from Ethiopia were analysed using amplified fragment length polymorphism (AFLP) markers to assess their genetic diversity. The estimated genetic distance for pairwise accessions ranged from 0.29 to 0.71, indicating the prevalence of genetic diversity in both exotic and local collections. Collections from different parts of Ethiopia, especially those from central and northwest regions revealed considerable variations between and within their geographic locations and thus can be used to create useful genetic variations. Cluster analysis categorized the accessions into 13 clusters, each consisting of one to 14 accessions. Two introduced accession were individually clustered, showing their greater divergence. Hence, AFLP analysis was found effective in discriminating the accessions and in selecting suitable parents for future genetic improvement.

8.2 Introduction

Cultivated linseed (*Linum usitatissimum* L.) is a self-pollinated diploid crop with the somatic chromosome number of 2n = 30 (Lay and Dybing, 1989). Cultivars of linseed are thus regarded as homozygous, and individual plants are considered homozygous (Chen *et al.*, 1998). The genus *Linum* belongs to the family *Linaceae* and comprises of about 200 species, which are mostly distributed in northern hemisphere (Luhs and Friedt, 1994). The haploid chromosome number in the genus *Linum* shows wide ranges (n = 8, 9, 10, 12, 14, 15 and 16), n = 9 and n = 15 being the most common ones. Some cytological evidence indicate that n = 15 species are secondary balanced polyploids, perhaps originating from a wild ancestor with a basic chromosome number of x = 8 (Luhs and Friedt, 1994). These authors further indicated that *L. usitatissimum* is the only species of agricultural importance, though the closely related *L. angustifolium* (2n = 30) had also been cultivated in some areas. Linseed is currently an important oil crop in the subtropics and temperate regions of the world (Luhs and Friedt, 1994). The

extracted oils are mostly used for paints, varnish and other industrial uses. Recently, its potential as a highly nutritive source, rich in linolenic acid and lignan, imparting appreciable health benefits has been emphasized (Payne, 2000).

Linseed, after niger seed (*Guizotia Abyssinica* Cass) is the second most important oilseed crop in the highlands of Ethiopia, with the largest areas of cultivation in the south-eastern and central parts of the country (Adefris *et al.*, 1992; Adugna, 2000). Existing varieties of linseed, in Ethiopia, have been developed for a number of traits including high seed set, oil yield, variation in maturity period, increased adaptability to different growing environments, enhanced resistance to pathogens, pests and other biotic and abiotic stresses (Adugna, 2000). Breeding improved cultivars of linseed is generally based on selection of germplasm and crosses based on the standard pedigree method. However, parental genotypes are selected on the basis of phenotypic performance and this may not be sufficient to determine genetic constitution. This limits the likelihood of developing successful new varieties. Thus, marker-assisted selection may help to overcome such problems.

Genetic characterization of germplasm can reveal the extent of genetic relatedness among accessions by estimating their genetic diversity and is also useful in the conservation of genetic resources. The analysis of genetic diversity among linseed accessions is important for cultivar identification and seed certification. Under the International Union for the Protection of New Varieties of Plants (UPOV, 1991), plant breeders' rights (PBR) are based on criteria of distinctiveness, uniformity and stability (DUS) of genotypes. Linseed cultivars are currently distinguished from each other on the basis of a number of morphological characters. However, expression of traits, such as days to flowering, maturity, plant height, number of branches and seed yield are significantly influenced by the environment and present problems for consistent identification. Moreover, uncontrolled pollen contamination, vectored by insects can add to the difficulties for assessments of genetic uniformity and stability.

Biochemical and molecular markers such as storage proteins and DNA fingerprints are not affected by environmental factors, and provide an important means to support cultivar identification (Kumer, 1999). Such markers are accepted as supplementary

characters in many countries (Roldan-Ruiz *et al.*, 2001). Genetic marker analysis is, therefore, important for the assessment of DUS criteria and the establishment of PBR, which is essential in the derivation of new varieties and seed purity certification.

Molecular markers are already being used to maintain germplasm collections (Barret and Kidwell, 1998). The rate of breeding improved varieties should improve with an increased genetic knowledge of the available germplasm. A disadvantage of the use of molecular markers is that specialist knowledge is required, as well laboratory equipment and chemical supplies making these markers more expensive than the morphological descriptors.

Amplified fragment length polymorphisms (AFLPs), was initially developed by Zabeau and Vos (1993) and has proved an efficient polymerase chain reaction (PCR) based technique to generate a large number of polymorphic DNA fragments (Altaf Khan *et al.*, 2002). It involves three basic steps, namely (a) digestion of DNA and ligation of oligonucleotide adapters, (b) amplification of restriction fragments, and (c) gel analysis of the amplified fragments. PCR amplification of restriction fragments is achieved by using adapters ligated to restriction site sequences as target sites for primer annealing. Selective amplification is achieved by using primers that extend into the sequence of the restriction fragments, thus amplifying only those fragments in which the primer extensions match the nucleotides flanking the restriction site. AFLP fingerprints are highly reproducible and have been used as a tool for evaluating genetic diversity (Majer *et al.*, 1996; Guthridge *et al.*, 2001). AFLP markers are reproducible even against the background of different combinations of Taq DNA polymerases and buffers (Powell *et al.*, 1996). However, the quantities of higher molecular weight fragments (< 400 base pairs, bp) is reduced when using plant DNA of poor quality (Guthridge *et al.*, 2001). The capacity of AFLP analysis to detect thousands of independent genetic loci with reasonable cost and time requirements makes it an ideal marker system for a wide array of genetic investigations (Maughan *et al.*, 1996).

An AFLP genetic linkage map of linseed was used to identify two quantitative trait loci (QTL) in independent linkage groups that exhibit a major effect on resistance to Fusarium wilt, a deadly disease of linseed (Spielmeyer *et al.*, 1998). They generated

213 marker loci covering approximately 1400 cM (centi-Morgan) of the linseed genome (n =15) with a mean spacing of 10 cM and comprising 18 linkage groups. This research illustrated the potential of AFLP as a powerful and fast method to generate moderately saturated linkage maps, allowing molecular analysis of traits that show oligogenic patterns of inheritance. Similarly, Hausner *et al.* (1998) developed co-dominant PCR/RFLP based markers for the linseed rust resistance alleles. These markers were used to confirm the presence of important alleles in certain recently released Canadian cultivars. This evidence adds to the growing support for the use of marker-assisted selection (MAS) and the introduction of new resistance genes for diseases like rust, Fusarium wilt and other important traits in a linseed breeding program. Therefore, the aim of this study was to analyse and determine the genetic variation among 60 accessions of linseed using AFLP DNA markers. The potential of discriminating between linseed genotypes using AFLP profiling was investigated by comparing individual accessions. Furthermore, we attempted to demonstrate whether AFLP markers would identify greater variation for genetic diversity assessment, and cultivar identification in comparison to morphological description and thereby enhance the genetic improvement of linseed in breeding programs.

8.3 Materials and Methods

Plant materials

The sixty accessions analysed in this study were selectively taken from the Highland Oil Crops Research Program of Holeta Research Centre in the Ethiopian Agricultural Research Organization to represent the diversity of linseed germplasm in Ethiopia. Fifty-three accessions were collected from different regions of Ethiopia and Eritrea (Fig. 3.1), with the remaining seven introductions from Europe, USA and Canada (Table 8.2). Four varieties (Chilalo, CI-1525, CI-1652 and Belay 96) were nationally released cultivars adapted to different environments across Ethiopia.

DNA Extraction

DNA was extracted from 1.5 month-old plant tissues (tips of stem including leaves) grown under glasshouse conditions at the University of Free State (South Africa) following the modified monocot extraction protocol (Edwards *et al.*, 1991), as presented in Appendix 2. The plant tissue was harvested from three random plants per

accession. The tissue was kept on ice until it was ground to a fine powder in a mortar and pestle after the addition of liquid nitrogen. Extraction buffer (10 ml) (1 M Tris-HCl pH 8.0, 0.25 M EDTA and 20% SDS) preheated at 65°C was added to each powdered sample along with 1 ml CTAB (Cetyl triethyl ammonium bromide) buffer (10% w/v) and 2 ml 5 M NaCl. The samples were incubated at 65°C in a water bath for an hour, with agitation every 10 to 20 minutes. Chloroform-isoamyl alcohol (24:1 v/v) (10 ml), was added and gently mixed, followed by centrifugation for 15 minutes at 10 000 rpm at room temperature. Chloroform extractions were repeated until the interfase was visibly free of debris. The DNA was precipitated by the addition of 100% cold ethanol was added in a 1:2 (v/v) and kept at 4°C overnight.

The precipitated DNA was spooled with a sterile Pasteur-pippette and washed twice in approximately 1 ml of 70% ethanol. Thereafter, the DNA was dissolved in 250 ml double distilled sterile water and stored at 4°C. DNA concentration and purity was determined using a spectrophotometer at 260 nm. The quality of genomic DNA was assessed by agrose electrophoresis on 200 ng DNA (0.5 g agarose, 50 ml 0.5x TAE buffer (40 mM Tris acetate 1 mM EDTA, pH 8.0), 0.5 µl Ethidium bromide) for approximately 45 minutes at 80 volts. The integrity and concentration of DNA was visualized and confirmed against the standard DNA (Marker III) under UV light. The DNA samples were diluted to a working concentration of 250 ng/µl and stored at 4°C.

Restriction endonuclease digestion and ligation of adapters

The AFLP reactions were conducted on DNA samples from individual accessions according to kit instructions (Gibco-BRL). RNase digestion was performed on each sample by the addition of 1 µl (ng/µl) RNase and incubated at 42°C for 30 minutes to remove RNA (Viljoen, 1996). Single genomic DNA digests were carried out to confirm the purity of DNA at 37°C for 30 minutes, as follows: 0.2 µl *Eco*RI (units), 2.5 µl DNA [250 ng], 10x buffer, 6.3 µl ddH_2O (sabax water), and 0.5 µl *Mse*I (units), 2.5 µl DNA [250 ng], 1 µl 10x buffer, 0.1 µl BSA, 5.9 µl ddH_2O. After mixing, the DNA samples were incubated for 3 to 4 hours at 37°C. Electrophoresis was performed, as stated to determine the extent of DNA digestion. Double digests were conducted by adding 2 µl *Eco*RI/ *Mse*I (units), 2.5 µl DNA [250 ng], 5x reaction

buffer and 15.5 µl ddH_2O. Samples were incubated for two hours at 37°C, after which the restriction enzymes were by incubation at 70°C for 10 minutes. Ligation of *Eco*RI and *Mse*I adapters was performed by mixing 25 µl of double digested DNA, 24 µl ligation solution (*Eco*RI/ *Mse*I adapters, 0.4 mM ATP, 10 mM Tris-HCl (pH 7.5) 10 mM Mg-acetate, 50 mM K-acetate) and 1 µl T4 DNA ligase (units). After incubation at 20°C for two hours, the ligated DNA was diluted 1:10 in TE buffer (10 mM Tris-HCl (pH 8.0) 0.1 mM EDTA). Detailed procedure of restriction digestion, ligation and PCR is shown in Appendix 3.

Polymerase chain reaction

Pre-selective PCR was performed in 51 µl with 5 µl diluted template DNA (1:10 dilution), 40 µl pre-amplification primer mix, 5 µl 10x PCR buffer (200 mM Tris-HCl (pH 8.4), 15 mM $MgCl_2$, 500 mM KCl) and 1 µl Taq DNA polymerase (units). A touch down Hybaid Thermal Cycler PCR was used to perform the reaction for 20 cycles at: 94°C for 30 seconds (s), 56°C for 60 s and 72°C for 60 s. Amplification was confirmed by running 12 µl on a 1% agarose gel. Pre-selective PCR product was diluted 1:50 in TE buffer.

Table 8.1. List of adapters and primer pairs tested in the study for selective reaction in AFLP amplification, 2001-2002

*Mse*I – adapter 5'-GACGATGAGTCCTGAG-3' 3'-TACTCAGGACTCAT-5'		*Eco*RI – adapter 5'-CTCGTAGACTGCGTACC-3' 3'-CATCTGACGCATGGTTAA-5'	
*Mse*I – primer	Code	*Eco*RI – primers	
*Mse*I + CAT	M2	*Eco*RI + ACA (Fam)	*Eco*RI + ACC (Ned)
*Mse*I + CTG	M3	*Eco*RI + ACA (Fam)	*Eco*RI + ACC (Ned)
*Mse*I + CTT	M4	*Eco*RI + ACA (Fam)	*Eco*RI + ACC (Ned)
*Mse*I + CTA	M5	*Eco*RI + ACA (Fam)	*Eco*RI + ACC (Ned)
*Mse*I + CAG	M6	*Eco*RI + ACA (Fam)	*Eco*RI + ACC (Ned)
*Mse*I + CAC	M7	*Eco*RI + ACA (Fam)	*Eco*RI + ACC (Ned)
*Mse*I + CTC	M8	*Eco*RI + ACA (Fam)	*Eco*RI + ACC (Ned)

Selective PCR-reaction was performed in a 20 µl PCR reactions containing 5 µl diluted pre-selective DNA, 5.5 µl Mix 1 (4.5 µl *Mse*I primer (6.7 ng/µl, dNTP's) and 1 µl *Eco*RI Fam and Ned labelled primer) (250µl) and 9.5 µl Mix 2 (2 µl 10x PCR buffer, 0.1 µl Taq DNA polymerase (units), 7.4 µl sterile dd H_2O). Reactions were performed using the following thermocycle profile: One cycle was performed at 94°C for 30 s, 65°C for 30 s, 72°C for 2 minutes. Thereafter annealing temperature was reduced by 0.7°C for 12 consecutive cycles followed by 23 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 2 minutes. The PCR product was prepared for capillary electrophoresis by the addition of 5 µl Fam labelled PCR product, 5 µl Ned labelled PCR product, 24 µl deionised Formamide and 1 µl Rox standard (GS-500) denatured at 94°C for 5 minutes with quick cooling in ice slurry. Capillary electrophoresis was performed using a Perkin Elmer Prism 310 Automated capillary sequencer (PE Biosystems). A total of seven pairs of primer combinations were screened and two pairs were selected based on the number of fragments amplified per primer pairs (Table 8.1). *Eco*RI primers (PE Biosystems) were labelled with Ned and Fam.

Data analysis

Each accession was analysed with two primer pairs and the primer extensions used were *Eco*-ACA/ *Mse*-CAT and *Eco*-ACA/ *Mse*-CTT (Table 8.1). The size of AFLP fragments was assessed by using GeneScan® Analysis Software (PE Biosystems). The presence and absence of AFLP fragments was recorded and converted into binary data (0 and 1). In order to summarize the relations between accessions, Euclidean genetic distances were calculated, using the available Number Cruncher Statistical System, NCSS 2000 (Hintze, 1998) and cluster analysis was performed and visualized using cophenetic correlation of the same statistical software. Cluster analysis was based on similarity matrices, using the unweighted pair group method with arithmetic mean (UPGMA), and relationship between accessions were visualized as dendrograms. The use of a simple matching similarity coefficient (Sokal and Michener, 1958) are reported for binary data analysis of dominant markers such as AFLP and for self-pollinating crops, whereas Nei and Li coefficient (Nei and Li, 1979) or the algebraic equivalent Dice coefficient (Dice, 1945) are suggested for co-dominant markers. But, Fuentes *et al.* (1999) found similar results by comparing both indices in the genetic diversity of rice genotypes using RAPD, AFLP and isozyme.

8.4 Results

Of the seven pairs of primers screened (Table 8.1), *Eco*-ACA/ *Mse*-CAT and *Eco*-ACA/ *Mse*-CTT resulted in a greater number of amplification products. Primers *Eco*-ACA/ *Mse*-CTG, *Eco*-ACA/ *Mse*-CTA and *Eco*-ACA/ *Mse*-CAG also produced reasonable amplification, unlike *Eco*-ACA/ *Mse*-CAC and *Eco*-ACA/ *Mse*-CTC that resulted in poor amplification. A total of 10377 fragments were generated by the two AFLP primer pairs (Table 8.2) for all accessions. For the primer combination *Eco*-ACA/ *Mse*-CAT approximately 86 bands were scored ranging from 40 to 409 bp, whereas for the primer combination *Eco*-ACA/ *Mse*-CTT 87 fragments were scored between 40 and 408 bp. There was no complete sharing of AFLP fragments for any two accessions, indicating genotypic differences. Fam labelled *Eco*-ACA)/ *Mse*-CTT primer generated an average of 60 AFLP fragments per accessions, followed by another Fam labelled *Eco*-ACA/ *Mse*-CAT with 49 fragments though a few accessions, such as number 33 and 51 repeatedly revealed considerably lower numbers of fragments (Figure 8.1).

The computed genetic distance estimates for all pairwise combinations of the 60 accessions is presented in Appendix 4. The overall genetic distance of all pairs of combinations (N = 1770) varied from 0.29 to 0.71. The highest genetic distance was found between accession numbers 22 and 57, whereas the smallest genetic distance was measured between accession numbers 33 and 51. These latter two accessions also had the lowest number of fragments (Table 8.2). The second highest range of genetic distances (0.70 to 0.71) was obtained for five pairs of accessions (25 and 57, 20 and 45, 23 and 59, 37 and 57, and 45 and 57). More than 50% of the pairs of accessions displayed greater genetic diversity values than the mean value of 0.57. There was also highly significant ($P < 0.001$) difference between the pairs of accessions for these values. Nevertheless, accession pairs, like 9 and 10 and 11 and 12 exhibited one of the smallest genetic distances, indicating their similarity in genetic bases. All of them were collected from different regions of Ethiopia except accession no. 9, which was acquired from Eritrea (ex-province of Ethiopia).

Table 8.2. Summary of 60 linseed accessions, their collection areas and AFLP generated fragments using four AFLP primers at UFS, 2002

No.	Accession	Collection area	M2 Fam	M2 Ned	M4 Fam	M4 Ned	Sum	Mean
1	10002	Shewa/ Ambo	16.00	4.00	63.00	43.00	126.00	31.50
2	10005	Sidamo/ Bore	80.00	15.00	61.00	29.00	185.00	46.25
3	10007	Kefa/ Omonada	47.00	8.00	46.00	24.00	125.00	31.25
4	10008	Gonder/ Fogera	89.00	10.00	62.00	32.00	193.00	48.25
5	10010	Hararge/ Chiro	14.00	6.00	50.00	24.00	94.00	23.50
6	10022	Welo/ Kalu	69.00	17.00	55.00	29.00	170.00	42.50
7	10026	Gojam/ Dambecha	27.00	45.00	54.00	23.00	149.00	37.25
8	10037	Sidamo/ Bore	35.00	8.00	67.00	44.00	154.00	38.50
9	10039	Eritrea/ Mendefera	3.00	9.00	53.00	28.00	93.00	23.25
10	Chilalo	Arsi/ n.a.	6.00	6.00	60.00	40.00	112.00	28.00
11	10041	Bale/ Adaba	52.00	46.00	6.00	3.00	107.00	26.75
12	10046	Welega/ Bedele	37.00	35.00	5.00	7.00	84.00	21.00
13	10060	Gonder/ Chera	37.00	42.00	62.00	27.00	168.00	42.00
14	10061	Gamo-Gofa/ M-Abay	40.00	45.00	74.00	31.00	190.00	47.50
15	10068	Gojam/ Bahir-Dar	66.00	38.00	57.00	37.00	198.00	49.50
16	10080	Hararge/ Habru	98.00	25.00	65.00	40.00	228.00	57.00
17	10085	Hararge/ Kuni	52.00	53.00	62.00	27.00	194.00	48.50
18	10104	Shewa/ Dendi	52.00	48.00	78.00	34.00	212.00	53.00
19	10109	Shewa/ Tach-Bet	79.00	56.00	76.00	38.00	249.00	62.25
20	CI-1525	Europe/ n.a.	23.00	57.00	107.00	35.00	222.00	55.50
21	10111	Shewa/ n.a.	35.00	59.00	42.00	3.00	139.00	34.75
22	10118	Shewa/ Ambo	81.00	53.00	88.00	35.00	257.00	64.25
23	10119	Shewa/ Chelia	83.00	27.00	71.00	47.00	228.00	57.00
24	10120	Sidamo/ Adola	85.00	20.00	89.00	41.00	235.00	58.75
25	10125	Tigray/ Inderta	67.00	38.00	74.00	44.00	223.00	55.75
26	10138	Welega/ Abe-Dongoro	69.00	14.00	58.00	23.00	164.00	41.00
27	10144	Welo/ Dese-Zuria	91.00	52.00	62.00	21.00	226.00	56.50
28	10159	Gonder/ Dabat	38.00	41.00	61.00	25.00	165.00	41.25
29	10162	Gonder/ Dabat	90.00	15.00	57.00	32.00	194.00	48.50
30	CI-1652	Europe/ n.a.	70.00	47.00	54.00	24.00	195.00	48.75
31	10169	Gonder/ Chera	67.00	58.00	64.00	36.00	225.00	56.25
32	10176	Gojam/ Guangua	14.00	73.00	87.00	16.00	190.00	47.50
33	10179	Shewa/ Alem-Gena	6.00	5.00	7.00	4.00	22.00	5.50
34	10185	Shewa/ Chelia	35.00	50.00	55.00	6.00	146.00	36.50
35	10192	Shewa/ Dendi	64.00	46.00	42.00	22.00	174.00	43.50
36	10197	Shewa/ Ambo	14.00	30.00	47.00	23.00	114.00	28.50
37	10204	Welo/ Bati	45.00	54.00	82.00	46.00	227.00	56.75
38	10006	Ilubabor/ Gore	70.00	51.00	61.00	41.00	223.00	55.75
39	10042	Tigray/ Lay-Machew	77.00	60.00	60.00	26.00	223.00	55.75
40	Belay 96	Shewa/ Holeta	66.00	52.00	64.00	41.00	223.00	55.75
41	10047	Ilubabor/ Bedele	75.00	37.00	45.00	16.00	173.00	43.25
42	10062	Bale/ Robe	37.00	16.00	49.00	43.00	145.00	36.25
43	10235	Gonder/ n.a.	63.00	49.00	69.00	14.00	195.00	48.75
44	10236	Gonder/ n.a.	32.00	54.00	69.00	27.00	182.00	45.50
45	10064	GamoGofa/BakoGazer	87.00	52.00	83.00	43.00	265.00	66.25
46	10072	Gojam/ Dejen	82.00	57.00	48.00	29.00	216.00	54.00
47	10073	Gojam/ Maychekel	77.00	55.00	50.00	45.00	227.00	56.75
48	10246	Ethiopia/ n.a.	30.00	38.00	52.00	30.00	150.00	37.50
49	10248	Ethiopia/ n.a.	18.00	26.00	53.00	30.00	127.00	31.75
50	10250	Ethiopia/ n.a.	21.00	42.00	48.00	48.00	159.00	39.75
51	10252	Ethiopia/ n.a.	13.00	9.00	9.00	6.00	37.00	9.25
52	10254	Ethiopia/ n.a.	16.00	31.00	87.00	31.00	165.00	41.25
53	10256	Ethiopia/ n.a.	17.00	36.00	62.00	25.00	140.00	35.00
54	10258	Ethiopia/ n.a.	5.00	36.00	75.00	23.00	139.00	34.75
55	10260	Ethiopia/ n.a.	13.00	21.00	86.00	18.00	138.00	34.50
56	Omega	USA/ North Dakota	10.00	23.00	61.00	11.00	105.00	26.25
57	R12-N1266	Canada/ Saskatoon	85.00	64.00	55.00	18.00	222.00	55.50
58	R12-M20G	Canada/ Saskatoon	72.00	57.00	35.00	10.00	174.00	43.50
59	CDC-1747	Canada/ Saskatoon	88.00	65.00	110.00	18.00	281.00	70.25
60	CDC-1747VG	Canada/ Saskatoon	9.00	8.00	49.00	25.00	91.00	22.75
	Sum		2939.00	2194.00	3583.00	1661.00	10377.00	2594.25
	Mean		48.98	36.57	59.72	27.68	172.95	43.24
	SE		47.52	35.45	57.82	26.82	167.42	41.86

The first pair of accession (22 and 57) that showed the highest diversity estimate (0.71) was acquired from local and exotic sources (Table 8.2). Accession no. 22 was collected from Ambo/ Ethiopia, while 57 was introduced from Canada in 1990 (Adugna, 2000). Similarly, the next highest diversity estimate was observed between the same exotic genotype and another locally collected accession (No. 25) from northern Ethiopia. The same was true with the third divergent pair (No. 20 and 45), indicating the presence of immense genetic diversity between the Ethiopian linseed germplasm and the exotic ones, specifically those from Canada.

The dendrogram, which was constructed on the basis of the generated AFLP data, divided the 60 accessions into 13 clusters (Table 8.3 and Fig. 8.2), consisting of one to 14 accessions each. Cluster I consisted of two accessions both were introduced from Canada. Similarly, clusters III, V and X comprised of two accessions, which were collected from different parts of Ethiopia. Cluster II and VI each contained five accession (one exotic and four Ethiopian germplasm each). Cluster IV had three Ethiopian accessions, while clusters VIII and XI consisted of six Ethiopian accessions each except acc. no. 60 in cluster VIII, which was introduced from Canada. Cluster IX was the largest with 14 accessions, followed by cluster VII that had 11 collections. Accessions in these two clusters were locally gathered from different geographical regions of Ethiopia except acc. no. 9 that was collected from Eritrea. Cluster XII and XIII comprised one accession each, and both accessions were exotic introductions. Acc. no. 20 was originally introduced from Europe and it was commercially released in Ethiopia since 1984 due to its wilt-resistance and high yield (Adefris *et al.*, 1992). In short, this result shows that clustering was not necessarily based on the geographic origins of the accessions but on genetic similarity of the materials.

Table 8.3. Cluster distribution of 60 linseed accessions based on the AFLP analysis, 2002

Cluster	No. of acc.	Name of accessions and their collection areas, as abbreviated* in the parenthesis
I	2	57, 58 (Saskatoon/ Canada)
II	5	52, 53, 54, 55 (Eth), 56 (USA)
III	2	47, 46 (G-NW)
IV	3	13, 28 (Gr-NW), 14 (GG-SW)
V	2	32 (G-NW), 34 (S-C)
VI	5	7 (G-NW), 18, 35 (S-C), 30 (Europe), 31 (Gr-NW)
VII	11	15 (G-NW), 16, 17 (H-E), 19, 22, 23 (S-C), 24 (S-S), 25 (T-N), 26 (W-W), 27 (W-N), 29 (Gr-NW)
VIII	6	11 (B-S), 12 (W-W), 21, 33, 51 (S-C), 60 (Canada)
IX	14	1 (S-C), 2, 8 (S-S), 3 (K-SW), 4 (Gr-NW), 5 (H-E), 6 (W-N), 9 (Eritrea), 10 (A-S), 36 (S-C), 42 (B- S), 48, 49, 50, (Eth)
X	2	39 (T-N), 41 (I-W)
XI	6	37 (W-N), 38 (I-W), 40 (S-C), 43 (T-N), 44 (Gr-NW), 45 (GG-SW)
XII	1	20 (Europe)
XIII	1	59 (Canada)

* G-NW = Gojam-North West; Gr-NW = Gonder-North West; GG-SW = Gamo-Gofa-South West; S-C = Shewa-Centre; H-E = Hararge-East; I-W = Ilubabor-West; T-N = Tigray-North; B-S = Bale-South; Sidamo-South; Arsi-South; K-SW = Kefa-South West; W-N = Welo-North; W-W = Welega-West; A-SE = Arsi South-East, parts of Ethiopia; Eth = Ethiopia, specific location could not be traced.

8.5 Discussion

Some of the important parameters that determine the extent of genetic variability include distribution, sampling and plant characteristics, such as mode of reproduction, breeding behaviour and generation time (Bhat *et al.*, 1999). A wide distribution of linseed from the tropics to temperate regions of the world (Luhs and Friedt, 1994) has contributed to its diversity and geographic distribution may be the best predictor for variation, as endemic species often have low genetic diversity (Hamrick and Godt, 1990). Moreover, out-crossing (Mansby *et al.*, 2000), which is dependent on insect pollination may have contributed to the genetic variability of linseed. This is, specifically very relevant to the linseed germplasm enhancement program at Holeta Research Centre where both crop improvement and bee research and husbandry operate almost together within the same campus. Numerous bees are always observed visiting flowers of linseed during the flowering period (August to October) at Holeta. Although such practices contribute positively towards enriching the genetic diversity, they could also have adverse effects on some planned breeding activities and on the maintenance of genetic purity of seeds and other desirable stocks. Hence, some sort of

insect control measure (e.g., use of insect-proof cages) is necessary for some restricted breeding activities.

The genetic distances computed for all 60 accessions based on the squared Euclidean distance (Sneath and Sokal, 1973) ranged from 0.29 to 0.71 (Appendix 4). This wide range of genetic distance indicates a broad genetic base among these accessions, which encompasses both exotic and local collections. The need for desirable agronomic traits (high yield, pest and drought resistance, and early maturity) for different growing areas and the preference of consumers with respect to quality attributes such as seed colour, texture, oil content, and other aspects differs widely from region to region resulting in a regional based variation between accessions. For example, early maturing and drought resistance are preferred in the northern parts of Ethiopia, while medium to late maturing and parasitic weed (*Cuscusta epilinum*) and wilt (*Fusarium oxysporium*) resistance are required in the central, north-west and southern parts of the country. Both pests have been the major production constraints in Ethiopia (Adugna, 2000) and thus future research should focus on mapping and cloning of the resistant genes for this pests.

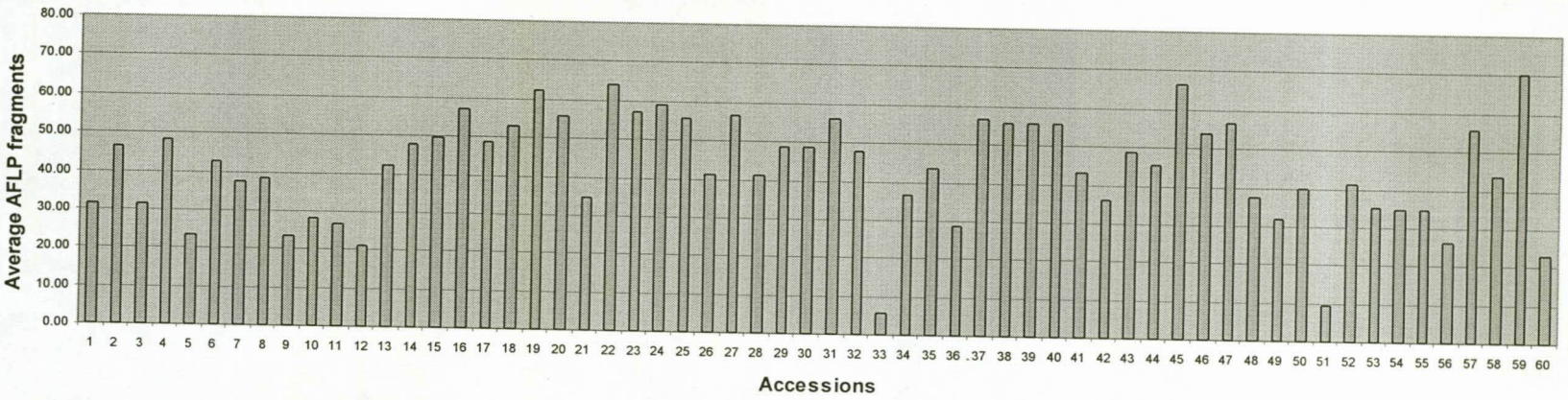


Fig. 8.1. Histogram showing averge AFLP fragments of the 60 linseed accessions

All the four commercial varieties (acc. no. 10, 20, 30 and 40) analysed in this study could be distinctly identified using AFLP markers in contrast to their phenotypic resemblance. For example, acc. no. 20 shared greater dissimilarity with other accessions and hence was not included in any group but unequivocally clustered away from the rest accessions, showing its clear divergence. Similarly, acc. no. 10, 30 and 40 were clustered in different clusters, indicating their distinct differences. These results indicate that AFLP analysis was sufficient to identify the accessions of linseed individually. Similarly, Guthridge *et al.* (2001) and Lombard *et al.* (2002) recently concluded that AFLP markers are effective in discriminating close-bred cultivars of perennial ryegrass and rapeseed inbred lines, respectively.

Moreover, Barret and Kidwell (1998) reported more pronounced efficiency of AFLP in diversity assessment of wheat cultivars from the Pacific Northwest. In fact, they concluded AFLP analysis as an efficient technology for assessing genetic diversity among wheat cultivars. AFLP markers were also reported to offer superior efficiency in terms of polymorphism rate in soybean (Maughan *et al.*, 1996), barley (Qi and Lindhout, 1997) and rice (Mackil *et al.*, 1996) when compared with other markers. However, it would be more interesting if time costs, monetary values, technical feasibilities and other constraints are equally reported along with their exciting results, especially under the African working conditions where we depend largely on imported equipment and supplies, and where various resources are very scarce and limiting.

The UPGMA-based dendrogram obtained from the combined data of AFLP revealed 13 close-knit clusters, comprising one to 14 accessions (Table 8.3 and Fig. 8.3). Two accessions showed greater dissimilarity with other accessions and hence were unequivocally clustered individually (XII and XIII) from the rest accessions, showing their clear divergence (Fig. 8.3). This dendrogram also reveals some interesting features of the studied accessions. For example, each of the eight clusters (II to XI) was consisted of similar accessions originally collected from the same or closer localities of Ethiopia, indicating the closer relationships of the accessions. Specifically, four accessions (acc. no. 52, 53, 54, and 55) in cluster II, each two accessions in clusters III and IV (13 and 28) and other accessions

collected from the same geographic region that were grouped within the same cluster were very similar. Similarity of accessions could be due to biased sampling during collections and also due to the exchange of seeds through the traditional farmer-to-farmer seed distribution system. In addition, out-crossing and other mechanical mingling during planting and harvesting operations may be the other causes of such a similarity. And this explains how AFLP could play effective roles in germplasm characterization for more efficient use of valuable genetic resources, money, time and other vital resources deployed to evaluate and conserve the germplasm resources. Nevertheless, the uses of co-dominant markers are suggested to assess the level of heterozygousity since linseed is considered as secondary polyploid (Luhs and Friedt, 1994).

On the other hand, collections from central Ethiopia (Shewa) were categorized under six different clusters (V, VI, VII, XIII, IX and XI). Likewise, accessions collected from northwest Ethiopia (Gonder, for example) were classified into five clusters (IV, VI, VII, IX and XI). The same was true with most of the other local accessions. This indicates the presence of ample genetic variability among the Ethiopian accessions that could be utilized in future breeding activities.

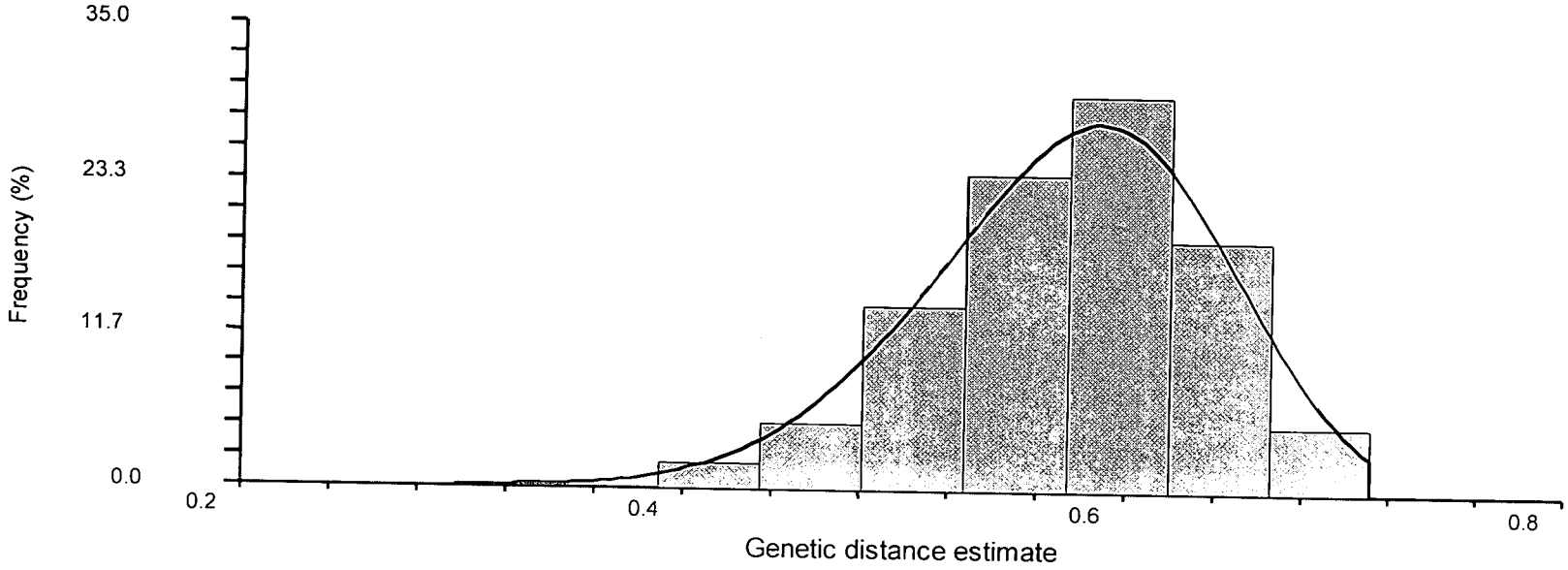


Fig. 8.2. Histogram depicting frequency distribution of genetic distances for all possible pairs of linseed accessions

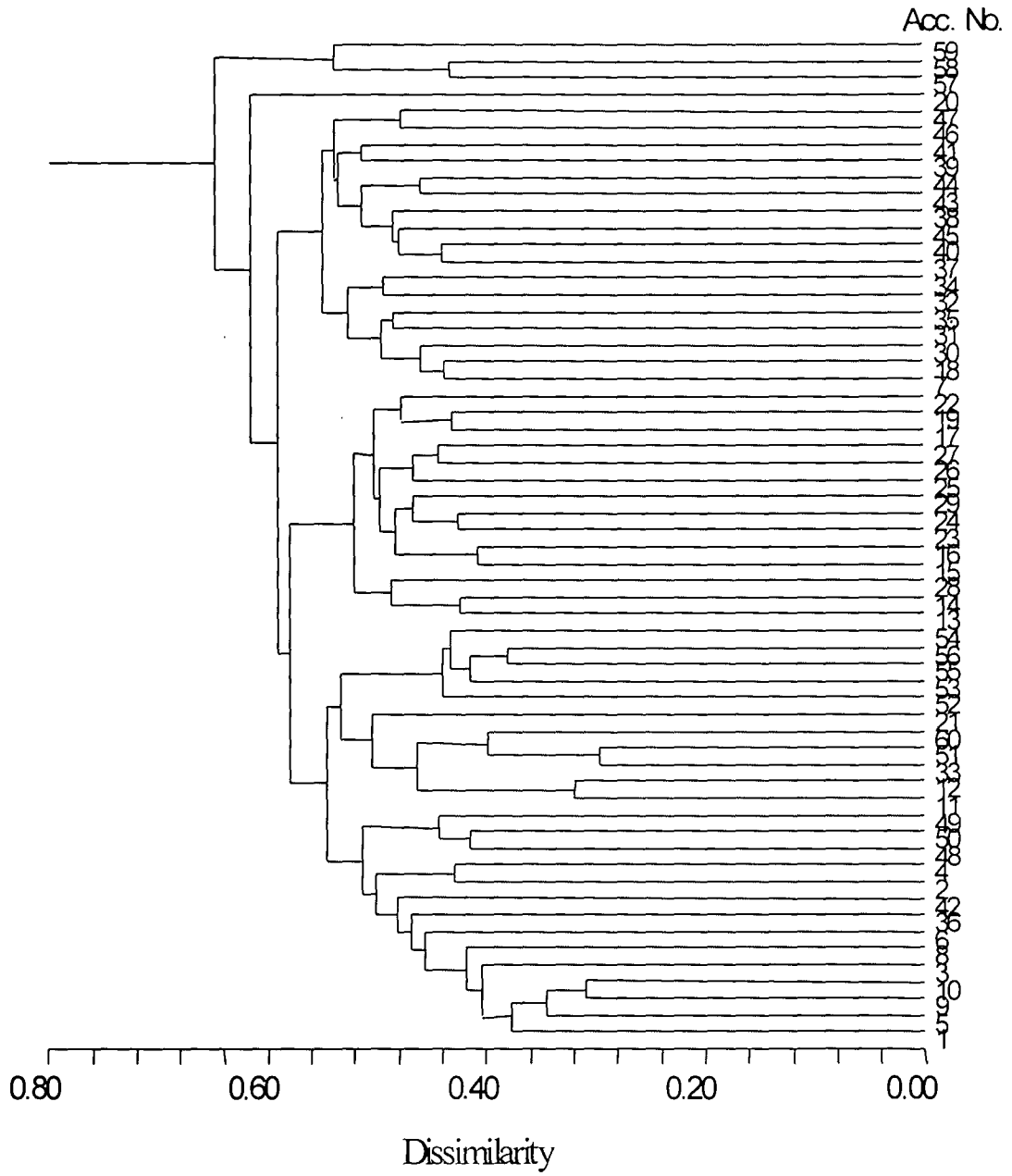


Fig. 8.3. Dendrogram indicating clusters of 60 accessions of linseed based on AFLP data, using the UPGMA clustering method

Some of the exotic accessions, like 30, 56 and 60, which were introduced from Europe, USA and Canada, respectively were grouped in three different clusters (II, VI and VIII) together with the Ethiopian collections. This similarity could be as the result of out-crossing (Mansby *et al.*, 2000), and trade and germplasm exchange programs between Ethiopia and Europe and North America that have been underway since long period of time. In general, the results of this study show that clustering was not necessarily based on the geographic origins of the accessions rather on their genetic constitutions. The presence of a wide range of genetic variation between the studied accessions is a good basis for future genetic improvement. Manjarrez-Sandoval *et al.* (1997) indicated that the evaluation of genetic diversity could provide predictive estimates of genetic variation among segregating progenies in pure line variety development programs. Similarly, such studies can also help in estimating the amount of heterosis (Barret and Kidwell, 1998). Thus, knowledge of the level and patterns in genetic diversity of linseed is useful for identifying diverse parental combinations to create segregating progenies with maximum genetic variability for subsequent selections. It is also useful to identify duplicate germplasm accessions.

8.6 Conclusions

AFLP markers showed a high level of DNA polymorphism among the analysed accessions of linseed. AFLP-based genetic distance estimates also revealed higher levels of genetic diversity among the accessions. Cluster analysis was useful to display the grouping patterns and relationships of individual accessions. This genetic information will contribute to linseed improvement by assisting in parental selections and by providing a predictive measure of important parameters, such as genetic variance and heterosis for future variety development programs. It is also vital for efficient management of germplasm. In future works, more statistical analyses, such analysis of molecular variance, bootstrapping and other latest statistical tools should be performed to consolidate the statistical significance of the findings.

CHAPTER 9

COMPARISON OF MORPHOLOGICAL AND AFLP MARKERS IN DIVERSITY ANALYSIS OF LINSEED

9.1 Abstract

Comparing the results of different methods of genetic diversity measures could give better judgment of differentiating important accessions for growers, germplasm curators and plant breeders. Data of 60 accessions of linseed from Ethiopia were used to assess their genetic diversity and compare morphological and amplified fragment length polymorphism (AFLP) methods. Analyses of genetic distance, principal component and cluster showed the presence of a wide range of diversity among the studied accessions. The mean for genetic distance estimates of the entire 1770 pairwise accessions was 0.6684 for morphology, while that of AFLP was 0.5734. These genetic distances were varied from zero to one for morphology in contrast to 0.29 to 0.71 of the AFLP. Substantial difference and very weak correlation (r = 0.076) was observed between the two genetic diversity matrices. Morphological and AFLP based clusters and their accompanying analyses also showed different hierarchical patterns of genetic diversity among the accessions. Despite their disparity, the two diversity measures were found independently adequate and useful for assessing and identifying the degree of relatedness and the overall patterns of genetic variations among the analysed linseed accessions.

9.2 Introduction

Diversity analysis of linseed (*Linum usitatissimum* L.) is an important component for efficient management and utilisation of its genetic resources, and for proper handling of the seed certification programs (Frankel, 1989; Blakeney, 2002). Accurate identification of genotypes or varieties is very useful during all the processes of breeding from initial parent selection to the final utilisation of cultivars in production schemes (UPOV, 1991). Thus, diversity analyses of all genotypes are an essential process for clear and sound identification of the genetic relatedness of the available

genetic resources. For proper choice of parents and subsequent crossing, breeders look for suitable descriptors or genetic markers.

Morphological or phenotypic descriptors have traditionally been used to distinguish one accession from the other. Although these types of agronomical characterisation provide useful information to the users, they are subjected to environmental influences, are time-consuming and they must be assessed during a fixed vegetative phase of the crop (Swanepoel, 1999). Conversely, the biochemical methods such as storage proteins and DNA characterisation techniques are accurate detectors, independent from the environment and the crop growing cycle (Kumar, 1999). However, they require specialist knowledge, laboratory equipment and chemical supplies that make them more expensive than the morphological descriptors. Therefore, using both morphological and biochemical characterisation can provide complementary advantages and this study tries to assess both techniques and their comparative benefits. To this end, the accessions were investigated for several agronomically important traits such as yield, oil content, maturity period, plant height and other useful characters besides the AFLP markers.

Analyses of plant genomes using DNA makers are allowing breeders to rapidly develop crop varieties with enhanced productivity (Altaf Khan *et al.*, 2002). Genome studies enable researchers to acquire valuable insights into how the crop genomes are organized and are also providing a number of practical applications, like variety identification through DNA fingerprinting; developing genetic maps that facilitate indirect selection of economic traits (e.g., disease resistance) without cumbersome screening; cloning of important genes; and evolutionary and phylogenetic studies (Altaf Khan *et al.*, 2002; Guthridge *et al.*, 2001). According to these authors, AFLP is a powerful, reliable, stable and rapid assay with potential genome mapping applications. Its analysis offers a rapid and efficient technique for detecting large numbers of DNA markers and should expedite plant gene isolation by positional cloning and the construction of high-density molecular linkage maps of plant genomes. The efficiency of generating AFLP markers appears to be much higher compared to other DNA markers. Most AFLP markers are dominant and show Mendelian inheritance (Maughan *et al.*, 1996).

The AFLP technique detects a much higher level of polymorphism than other DNA markers, and it is an efficient marker technology for the construction of genetic linkage maps (Kumar, 1999). Most AFLP markers correspond to unique positions on the genome and can be utilized for genetic and physiological mapping as each fragment is characterized by its size and primer combination required for amplification (Vos *et al.*, 1995). AFLP markers are generally randomly distributed throughout the genome. AFLP and Simple Sequence Repeats (SSR) DNA markers have proved to be efficient and reliable in supporting conventional plant breeding programs (Kumar, 1999; Guthridge *et al.*, 2001). Marker-assisted breeding or selection (MAS) has been offering the potential of deploying favourable gene combinations and for predicting better outcomes.

According to Arus and Morenzo-Gonzalez (1993) the most important properties for good quality markers are: (1) easy recognition of all possible phenotypes (homo- and heterozygotes) from all different alleles; (2) early expression in the development of the plant; (3) no effect on the plant morphology of alternate alleles at the marker loci; (4) low or null interaction among markers allowing the use of many at the same time in segregating populations. The recently established AFLP and simple sequence repeat (SSR also called microsatellite) techniques fulfil most of the requirements of good quality markers. Their high informative value, identification of polymorphisms, reproducibility as well as independence from the environment and their comparison with other markers have been demonstrated and reported across a wide range of plant species (Tohme *et al.*, 1996; Barret *et al.* 1998; Kumar, 1999; Guthridge *et al.*, 2001).

An AFLP genetic linkage map of linseed was used to identify two quantitative trait loci (QTL) on independent linkage groups with a major effect on resistance to Fusarium wilt, a deadly disease of linseed (Spielmeyer *et al.*, 1999). These authors illustrated the potential of AFLP as a powerful and fast method to generate moderately saturated linkage maps, allowing molecular analysis of traits like Fusarium wilt, that show oligogenic patterns of inheritance. Moreover, Hausner *et al.* (1998) developed the co-dominant PCR/RFLP based markers for the flax rust resistance alleles. Their result confirmed the presence of L^6 and L^9 rust resistant genes in several Canadian cultivars of linseed. This evidence indicates that

molecular markers are becoming useful in marker assisted selection and in introduction of new genes for resistance of diseases like rust, Fusarium wilt and other vital traits in linseed breeding programs. Hence, the main objectives of this study were to assess the genetic variation of 60 accessions of linseed using both morphological and AFLP markers. The potential for discrimination of accessions, using both methods were also compared to display the degree of relationships between the two methods.

9.3 Materials and Methods

Morphology

The materials and methods used for morphological diversity assessment were the same as those described in chapter five and list of accessions were shown in Tables 8.2 and 9.2. The list of morphological traits used in characterization is also presented in Table 9.1.

Table 9.1. List of morphological characters measured and their brief descriptions

Characters	Description
Plant height (PH)	Height above ground measured in centimetre at harvest
Days to flowering (DF)	Number of days from sowing to 50% flowering
Days to maturity (DM)	Number of days from sowing to 75% maturity
Primary branches per plant (PB/P)	Number of primary branches per plant at harvest
Secondary branches per plant (SB/P)	Number of secondary branches per plant at harvest
Number of bolls per plant (B/P)	Number of bolls per plant counted at harvest
Number of seeds per boll (S/B)	Number of seeds per plant counted after harvest
Seed yield per plant (SY/P)	Weight of seed yield in grams per plant
Total seed yield (TSY)	Weight of seed yield in grams per plot (g/m^2)
Thousand seed weight (TSW)	Weight of 1000 seeds in grams
Oil content (Oil%)	Percentage of oil content in dry seeds
Oil yield per plot (Oil yield)	Product of oil content and seed yield per plot (g/m^2)

AFLP markers

The materials and methods applied for AFLP analyses were identical to those presented in chapter eight (tables 8.1 and 8.2).

Data analysis

Data of both morphology and AFLP were scored for presence (1) and absence (0) and entered into a binary matrix representing the data profile of each accession. The morphological data were converted to binary data, taking their respective mean values as a benchmark. Values greater than their mean values were scored one and those equal or less than the mean were scored as zero. Measurements of genetic distance between individual accessions were calculated with the squared Euclidean distance (Sneath and Sokal, 1973), using the formula: $E^2_{ij} = \sum_k (x_{ki} - x_{kj})^2$, where k is the number of bands and x_{ki} and x_{kj} are the frequencies of occurrence (0 or 1) of the k^{th} band in individual i and j, respectively. Genetic distances between pairs of accessions, their principal components and cluster analyses were undertaken using the Number Cruncher Statistical System, NCSS 2000 (Hintze, 1998) statistical system for Windows. A dendrogram was constructed using the unweighted pair group method of arithmetic averages (UPGMA) with standard deviation scaling type.

9.4 Results

Morphological traits and AFLP fragments

The mean absolute values of the 12 morphological characters used in the characterization of the studied linseed accessions are presented in Table 9.2. These values were the average performance across four environments (two locations and two years). There was a significant ($P < 0.05$) difference between the accessions for all measured characters, indicating the presence of a wide range of diversity across the accessions for these traits, including plant height and days to maturity that ranged between 56-87 cm and 135-153 days, respectively. Similarly, oil content varied from 29-36% and oil yield ranged from 1443-3212 g/m^2. Given this diversity, all the 60 accessions were found to differ from each other in one or more individual characters at $P < 0.05$ significant level. This confirmed that some of the accessions were morphologically distinct. Investigation of the highest and lowest values of the morphological characters revealed that some accessions were frequently expressing trait values greater than their respective means (Figure 9.1, upper part). For example, acc. no. 3 was the greatest in almost 11 of its characters, followed by acc. no. 59.

Table 9.2. Mean of 12 morphological characters for 60 linseed accessions evaluated across four environments (UFS and HRC, 2000-2001)

	Characters											
Accession	HT	DF	DM	PB/P	SB/P	B/P	S/B	SY/P	TSY	TSW	Oil%	Oil yield
1. 10002	64	88	145	4	6	15	9	0.64	72	5.61	33.54	2398
2. 10005	58	83	145	4	6	18	9	0.59	68	5.45	31.20	2106
3. 10007	81	92	149	5	7	20	10	0.72	75	5.44	33.29	2480
4. 10008	70	91	144	4	7	20	10	0.73	74	5.69	33.54	2482
5. 10010	65	78	144	5	7	19	9	0.80	70	5.51	29.87	2091
6. 10022	83	91	153	4	5	13	9	0.68	72	6.11	32.42	2318
7. 10026	76	85	140	4	6	17	9	0.60	72	5.96	33.68	2425
8. 10037	75	76	144	4	6	19	9	0.69	70	5.56	33.80	2349
9. 10039	70	75	138	4	6	17	9	0.76	76	7.12	32.75	2473
10. Chilalo	85	81	144	4	5	16	9	0.71	88	6.78	34.58	3026
11. 10041	74	78	143	4	6	20	9	0.78	59	6.67	32.77	1934
12. 10046	64	74	141	4	6	17	9	0.63	62	6.25	32.03	1970
13. 10060	73	90	151	4	7	15	9	0.67	57	6.55	33.09	1886
14. 10061	64	84	143	4	7	17	10	0.59	64	5.34	30.79	1955
15. 10068	70	84	139	5	7	18	9	0.65	78	6.06	31.87	2486
16. 10080	61	75	136	5	6	18	9	0.73	55	6.05	31.58	1737
17. 10085	65	84	145	4	7	16	8	0.69	66	6.39	30.81	2034
18. 10104	68	85	147	4	7	19	9	0.77	72	6.30	30.61	2189
19. 10109	62	74	139	4	6	16	9	0.53	61	6.03	29.84	1820
20. CI-1525	87	82	152	4	7	19	8	0.85	82	7.28	35.62	2903
21. 10111	67	88	147	4	6	15	10	0.53	74	6.07	31.33	2303
22. 10118	71	84	147	5	7	18	8	0.64	72	5.98	31.21	2232
23. 10119	71	88	146	4	7	15	10	0.58	68	5.93	31.51	2127
24. 10120	77	78	139	4	7	17	9	0.78	75	6.33	33.85	2522
25. 10125	57	81	140	4	5	17	9	0.63	56	6.14	31.29	1737
26. 10138	63	86	142	4	7	17	9	0.56	70	5.46	29.93	2095
27. 10144	65	76	141	4	7	15	9	0.62	68	6.23	31.51	2143
28. 10159	70	72	149	4	6	14	9	0.68	67	6.10	33.84	2251
29. 10162	67	71	139	4	6	17	10	0.76	68	6.41	31.07	2097
30. CI-1652	82	81	151	4	7	19	9	0.80	86	6.85	35.00	2993
31. 10169	72	87	143	4	5	13	10	0.62	69	5.83	32.89	2270
32. 10176	66	88	146	4	6	14	9	0.54	69	5.56	29.42	2030
33. 10179	64	85	144	4	7	15	9	0.62	63	6.07	30.98	1952
34. 10185	72	84	141	4	6	17	10	0.71	66	6.55	30.45	2010
35. 10192	66	89	143	5	6	17	10	0.51	67	5.74	29.93	1991
36. 10197	67	88	146	4	6	14	9	0.55	71	5.93	31.25	2203
37. 10204	70	88	151	4	6	13	9	0.51	44	5.44	33.17	1443
38. 10006	62	73	137	5	8	24	8	0.86	71	5.62	30.85	2175
39. 10042	70	76	142	4	6	18	9	0.80	77	5.88	32.80	2509
40. Belay 96	83	81	144	4	6	18	10	0.81	94	6.07	35.03	3276
41. 10047	70	75	141	4	7	19	9	0.70	57	6.57	30.13	1718
42. 10062	81	76	144	4	6	16	9	0.79	70	6.92	33.99	2363
43. 10235	69	84	143	5	6	18	10	0.66	68	6.17	30.76	2077
44. 10236	59	72	135	4	5	16	8	0.68	63	6.35	33.28	2080
45. 10064	56	77	137	4	7	18	10	0.69	62	5.40	31.42	1948
46. 10072	60	83	143	4	6	16	9	0.59	68	5.66	31.26	2110
47. 10073	69	81	138	4	6	18	9	0.74	76	6.04	32.67	2483
48. 10246	65	75	140	4	6	19	8	0.84	67	6.24	31.24	2078
49. 10248	64	84	142	4	5	18	9	0.66	69	6.15	30.76	2123
50. 10250	64	87	148	5	6	18	9	0.61	59	5.70	30.01	1756
51. 10252	67	86	145	4	6	17	9	0.71	79	6.19	31.93	2523
52. 10254	65	86	143	4	5	16	9	0.54	67	6.14	31.09	2068
53. 10256	65	78	142	4	5	17	10	0.68	71	5.93	29.83	2103
54. 10258	65	85	144	4	5	18	9	0.63	76	6.01	31.98	2431
55. 10260	76	92	144	4	6	19	10	0.88	61	6.19	29.13	1763
56. Omega	85	82	143	5	6	22	9	0.99	80	6.11	32.55	2604
57. N1266	87	78	143	5	7	25	9	1.08	69	7.13	33.82	2317
58. M20G	86	84	148	4	6	22	9	0.96	86	7.39	33.42	2874
59. CDC1747	80	82	148	4	8	21	10	0.92	77	5.92	33.15	2536
60. CDCVG	80	83	146	4	6	17	9	0.85	90	7.04	35.88	3212
Mean	70.08	82.07	144	4.20	6.17	17.40	9.11	0.70	70	6.12	32.06	2243
CD 0.05	9.53	7.02	9.93	0.92	1.85	6.58	7.06	0.30	22	1.89	0.35	79.00

PH = Plant height; DF = Days to 50% flowering; DM = Days to maturity; PB/P = Primary branches/plant; SB/P = Secondary branches/plant; B/P = Bolls/plant; S/B = Seeds/boll; SY/P = Seed yield/plant (g); TSY = Total seed yield (g/m^2) TSW = Thousand seed weight (g); Oil yield (g/m^2)

Accessions 20, 30 and 58 were also on the next level with their nine characters above average, in contrast to accession 25 that was on the lowest extreme with its all characters below average, followed by acc. no. 12, 19, 46 and 52, unlike eight accessions (4, 18, 22, 24, 47, 56, 57 and 60) that displayed above average performance of traits.

The lower part of Figure 9.1 also shows the average number of AFLP fragments produced by the pairs of primers for each accession. Accession 59 was the highest with 70 AFLP fragments, followed by 45, 22 and 19. In contrast, acc. no. 33 and 51 showed the lowest fragments of below 10 despite their repeated tests. On the other hand, 15 other accessions (16, 18, 20, 23, 24, 25, 27, 31, 37, 38, 39, 40, 46, 47, and 57) generated above 50 AFLP fragments. Generally, 10 accessions (4, 18, 20, 22, 24, 30, 40, 57, 58 and 59) were observed to produce both above average morphological traits and AFLP fragments that were used in discriminating these accessions.

Genetic distances

The estimated genetic distances between pairs of accessions for morphological and AFLP analyses for the first 120 accessions were presented in Table 9.3. These distances were organised in descending order to demonstrate the rankings of both methods from the highest to the lowest. The distances of both methods were quite different in terms of values and rankings. For instance, the first four pairs of accessions that showed maximum genetic difference in morphological characters were 3 and 12, 3 and 19, 20 and 35, and 30 and 35. None of these pairs were present in the first four ranks for AFLP distances (Table 9.3). However, there were few pairs of accessions that showed relatively closer ranks between the genetic distances of morphology and AFLP. These pairs were shown on serial numbers 59^{th} and 20^{th}, 56^{th} and 5^{th}, 63^{rd} and 104^{th} and 103^{rd} and 27^{th} of morphological and AFLP distances, respectively. In fact, most of the pairs were far apart from each other and ranked quite differently under both systems of genetic distances, as partly shown in Table 9.3. The genetic distances of morphology for the total pairs (N = 1770) ranged between zero and one, while that of AFLP was varied from 0.2975 to 0.7112, with their mean of 0.6684 and 0.5731, respectively. Both genetic distances were not normally distributed (Fig. 2).

Table 9.3. Genetic distances (in descending order) between the first 120 pairs of accessions based on morphology and AFLP data analyses

Morphology							AFLP						
No.	Pairs		Distance	No.	Pairs		Distance	No.	Pairs		Distance	No.	Pairs		Distance
1	3	12	1.0000	61	50	59	0.9129	1	22	57	0.7112	61	4	57	0.6726
2	3	19	1.0000	62	53	57	0.9129	2	25	57	0.7065	62	11	20	0.6726
3	20	35	1.0000	63	1	57	0.8660	3	20	45	0.7018	63	15	59	0.6726
4	30	35	1.0000	64	2	10	0.8660	4	23	59	0.7018	64	16	59	0.6726
5	3	25	0.9574	65	2	40	0.8660	5	37	57	0.7018	65	21	45	0.6714
6	3	27	0.9574	66	2	57	0.8660	6	45	57	0.7018	66	27	57	0.6714
7	3	44	0.9574	67	2	59	0.8660	7	12	59	0.6994	67	41	59	0.6714
8	17	56	0.9574	68	3	17	0.8660	8	23	57	0.6958	68	19	53	0.6701
9	21	57	0.9574	69	3	28	0.8660	9	25	59	0.6958	69	3	59	0.6689
10	23	47	0.9574	70	3	32	0.8660	10	38	59	0.6958	70	15	57	0.6689
11	24	50	0.9574	71	3	34	0.8660	11	24	57	0.6946	71	29	57	0.6689
12	35	59	0.9574	72	3	37	0.8660	12	4	59	0.6934	72	4	20	0.6676
13	45	60	0.9574	73	3	49	0.8660	13	11	59	0.6934	73	21	57	0.6676
14	46	59	0.9574	74	3	53	0.8660	14	22	59	0.6934	74	27	59	0.6676
15	52	59	0.9574	75	4	12	0.8660	15	38	57	0.6922	75	40	57	0.6676
16	2	24	0.9129	76	4	19	0.8660	16	21	59	0.6910	76	47	53	0.6676
17	3	29	0.9129	77	5	6	0.8660	17	24	59	0.6910	77	13	57	0.6664
18	3	33	0.9129	78	5	13	0.8660	18	45	56	0.6910	78	22	56	0.6664
19	3	41	0.9129	79	5	28	0.8660	19	45	53	0.6898	79	22	60	0.6664
20	3	46	0.9129	80	5	34	0.8660	20	45	58	0.6898	80	23	53	0.6664
21	3	48	0.9129	81	5	60	0.8660	21	19	59	0.6886	81	24	31	0.6664
22	3	52	0.9129	82	6	29	0.8660	22	45	59	0.6886	82	37	59	0.6664
23	5	31	0.9129	83	6	38	0.8660	23	1	59	0.6874	83	45	54	0.6664
24	9	22	0.9129	84	6	41	0.8660	24	19	57	0.6874	84	13	59	0.6651
25	9	23	0.9129	85	6	43	0.8660	25	22	58	0.6874	85	24	53	0.6651
26	10	50	0.9129	86	6	45	0.8660	26	9	59	0.6861	86	28	57	0.6651
27	11	21	0.9129	87	6	48	0.8660	27	14	57	0.6861	87	42	57	0.6651
28	13	16	0.9129	88	8	18	0.8660	28	42	59	0.6861	88	46	59	0.6651
29	14	20	0.9129	89	9	50	0.8660	29	10	59	0.6849	89	20	57	0.6639
30	14	30	0.9129	90	10	14	0.8660	30	33	59	0.6849	90	23	32	0.6639
31	14	56	0.9129	91	10	22	0.8660	31	36	59	0.6849	91	22	53	0.6626
32	14	58	0.9129	92	10	23	0.8660	32	14	59	0.6825	92	37	53	0.6626
33	17	40	0.9129	93	10	35	0.8660	33	25	58	0.6825	93	16	53	0.6614
34	18	31	0.9129	94	10	43	0.8660	34	45	51	0.6813	94	27	53	0.6614
35	20	43	0.9129	95	10	45	0.8660	35	45	60	0.6813	95	6	59	0.6601
36	20	46	0.9129	96	11	15	0.8660	36	12	45	0.6800	96	12	38	0.6601
37	20	52	0.9129	97	12	59	0.8660	37	17	59	0.6800	97	18	59	0.6601
38	22	29	0.9129	98	13	38	0.8660	38	37	58	0.6800	98	20	38	0.6601
39	22	42	0.9129	99	13	39	0.8660	39	45	55	0.6800	99	20	39	0.6601
40	23	39	0.9129	100	13	56	0.8660	40	47	57	0.6800	100	23	56	0.6601
41	23	56	0.9129	101	14	40	0.8660	41	11	45	0.6788	101	26	59	0.6601
42	24	35	0.9129	102	14	47	0.8660	42	47	59	0.6788	102	27	45	0.6601
43	24	43	0.9129	103	14	57	0.8660	43	5	59	0.6776	103	38	53	0.6601
44	25	59	0.9129	104	14	59	0.8660	44	19	58	0.6776	104	1	57	0.6588
45	30	43	0.9129	105	14	60	0.8660	45	24	32	0.6776	105	11	37	0.6588
46	30	46	0.9129	106	15	29	0.8660	46	24	58	0.6776	106	16	32	0.6588
47	30	52	0.9129	107	15	42	0.8660	47	34	59	0.6776	107	22	54	0.6588
48	31	38	0.9129	108	16	23	0.8660	48	38	58	0.6776	108	27	58	0.6588
49	32	40	0.9129	109	16	31	0.8660	49	16	57	0.6763	109	30	57	0.6588
50	32	57	0.9129	110	17	39	0.8660	50	23	58	0.6763	110	47	56	0.6588
51	32	59	0.9129	111	19	59	0.8660	51	51	59	0.6763	111	50	59	0.6588
52	33	56	0.9129	112	20	25	0.8660	52	8	59	0.6751	112	12	37	0.6576
53	35	58	0.9129	113	20	32	0.8660	53	29	59	0.6751	113	16	56	0.6576
54	36	57	0.9129	114	20	34	0.8660	54	44	59	0.6751	114	19	55	0.6576
55	37	40	0.9129	115	20	37	0.8660	55	8	57	0.6739	115	20	47	0.6576
56	37	57	0.9129	116	20	49	0.8660	56	17	57	0.6739	116	27	56	0.6576
57	37	59	0.9129	117	20	50	0.8660	57	18	57	0.6739	117	43	57	0.6576
58	40	50	0.9129	118	20	53	0.8660	58	19	56	0.6739	118	47	55	0.6576
59	45	58	0.9129	119	22	28	0.8660	59	28	59	0.6739	119	47	58	0.6576
60	49	59	0.9129	120	22	40	0.8660	60	44	57	0.6739	120	2	59	0.6563
Mean = 0.6684; SE = 3.34; T-value = 200.37**; CV = 0.21								Mean = 0.5731; SE = 1.46; T-value = 392.38**; CV = 0.11							

N = 1770; ** = Significant at P < 0.01

The Pearson correlation between these morphological and AFLP distances was practically weak (r = 0.076) for row-wise comparison of the entire pairs (N =1770). This weak correlation shows the absence of a useful relationship between morphological and AFLP distances.

Principal component analysis

The results of principal component analysis for morphology and AFLP data are shown in Table 9.4. The former grouped its variables into 12 principal components, while the latter divided them into four principal components, accounting for the entire variability. The individual variability, which described morphology, ranged approximately from five to 10 percent, whereas the four AFLP principal components contributed almost 25% each. The first 11 eigenvectors of morphological characters explained 94.67% of the total variance. Out of these, the first 10 (those with eigenvectors greater than one) accounted for a cumulative value of 87.45%, indicating that all components contributed almost equally to the entire variability. However, characters such as days to flowering, bolls/plant, seeds/boll, 1000-seed weight and oil yield had relatively higher contributions to the total morphological variability. Likewise, *Eco*RI-ACC/ *Mse*-CAT and *Eco*RI-ACA/ *Mse*-CTT primers showed equal contributions to the total variability of the AFLP analysis. These similar in contributions of both morphological and AFLP components show the presence of a wide range of variability among the studied accessions.

Cluster analysis

The results of clusters analyses for morphological and AFLP data are given in Tables 9.5 and Figure 9.3. Based on the binary data of 12 morphological traits, the 60 accessions were grouped into 18 clusters, consisting of one to nine accessions each, while AFLP analysis clustered the same accessions into 13 groups, each comprising one to 14 accessions. Cluster I for morphology (Cluster I-M) comprised three accessions, whereas that of the AFLP (Cluster I-A) had two accessions (57 and 58) that were grouped in different classes by morphological clustering. Cluster II-M comprised two Ethiopian accessions, while cluster II-A had four local accessions and one introduction from USA.

Table 9.4. Eigenvectors and eigenvalues of 12 principal components for (a) 12 morphological characters and (b) four AFLP primers of 60 linseed accessions tested across four environments (UFS and HRS, 2000-2002)

(a) Morphological characters

	Eigenvectors											
Character	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
PH	-0.36	0.12	-0.16	-0.06	0.43	-0.46	-0.25	0.10	0.40	-0.03	0.47	0.04
DF	0.24	0.08	-0.58	-0.03	0.08	-0.10	0.11	-0.00	0.21	0.66	-0.29	-0.07
DM	-0.05	0.19	-0.51	-0.46	-0.13	-0.14	0.42	-0.12	-0.17	-0.48	0.01	0.12
PB/P	-0.02	-0.58	-0.12	0.06	-0.07	-0.39	-0.24	-0.64	-0.17	-0.01	-0.07	-0.02
SB/P	-0.06	-0.31	-0.08	-0.66	0.19	0.37	-0.47	0.20	-0.07	0.02	-0.15	-0.07
B/P	-0.21	-0.53	-0.01	-0.03	-0.04	-0.23	0.42	0.53	-0.27	0.19	0.15	-0.19
S/P	0.06	-0.11	-0.11	0.24	0.80	0.31	0.26	0.18	-0.21	-0.13	0.01	-0.11
SY/P	-0.44	-0.21	0.19	-0.01	0.05	0.08	0.34	-0.11	0.50	-0.03	-0.46	0.37
SY/PT	-0.34	-0.14	-0.31	0.12	-0.31	0.53	0.03	-0.18	0.27	0.02	0.37	-0.38
TSW	-0.21	0.26	0.40	-0.46	0.08	-0.02	0.24	-0.41	-0.17	0.43	0.16	-0.20
OC	-0.46	0.26	-0.06	0.11	0.03	-0.14	-0.21	0.09	-0.20	-0.13	-0.54	-0.54
OY	-0.44	0.16	-0.21	0.24	-0.06	0.13	-0.13	-0.01	-0.49	0.27	0.09	0.57
Eigenvalue	1.15	1.02	1.02	1.01	1.01	1.05	1.02	1.04	1.14	1.03	0.87	0.64
IVE (%)	9.60	8.47	8.45	8.44	8.45	8.74	8.53	8.63	9.52	8.61	7.22	5.33
CVE (%)	9.60	18.07	26.52	34.96	43.41	52.15	60.68	69.31	78.84	87.45	94.67	100.00

(b) AFLP primers

	Eigenvectors			
Primers	PC1	PC2	PC3	PC4
*Eco*RI-ACA/ *Mse*I-CAT	-0.61	0.33	0.39	0.61
*Eco*RI-ACC/ *Mse*I-CAT	-0.35	0.70	-0.31	-0.54
*Eco*RI-ACA/ *Mse*I-CTT	-0.48	-0.40	-0.75	0.21
*Eco*RI-ACC/ *Mse*I-CTT	-0.52	-0.49	0.44	-0.55
Eigenvalue	0.99	1.00	1.00	0.99
IVE (%)	24.99	25.02	25.01	24.98
CVE (%)	24.99	50.01	75.02	100.00

Note: PC = Principal components; IVE = Individual variability explained; CVE = Cumulative variability explained

Cluster III-M contained seven accessions exclusively collected from different parts of Ethiopia, and III-A also had two indigenous accessions collected from the northwest of Ethiopia. Cluster IV-M and IV-A consisted of three Ethiopian accessions each that were collected from different parts of Ethiopia. Similarly, cluster VI-M possessed three local accessions, while cluster IX-M, X-M and XII-M had two accessions each, collected from different regions of Ethiopia (Table 9.5). Cluster VIII-M had the largest number of Ethiopian accessions, like IX-A that also comprised 13 collections assembled from Ethiopia and one accession from Eritrea. Five morphological clusters (XIV-M, XV-M, XVI-M, XVII-M and XVIII-M) each had one accession, four of them being from indigenous sources. Likewise, two clusters of AFLP (XII-A and XIII-A) consisted of one exotic accession each (Table 9.5). In short, the two clusters of morphology and AFLP presented different pattern groupings and had little relationships in common.

9.5 Discussion

The primary objectives of the present study were to characterize the levels and patterns of genetic diversity among the accessions of linseed mainly collected from the highlands of Ethiopia using morphological and AFLP markers and to compare these two evaluation methods. These diversity analyses were based on the combined data of 12 morphological characters and four AFLP primers. For comparative analysis of diversity levels, the consideration of estimated genetic distance is very important, as it was well emphasized by other researchers (Roldan-Ruiz *et al.*, 2001). The overall mean of genetic distance for morphology was 0.6684, while that of AFLP was 0.5731 (Table 9.3). The highest genetic distances were mostly obtained from pairs of exotic and local collections, indicating their wide dissimilarity. Comparison of genetic diversity estimates between morphology and AFLP showed that the former ranged from zero to one, while the latter varied from 0.29 to 0.71. In the wider range of morphological distance, four pairs of accessions (3 and 12, 3 and 19, 20 and 35, 30 and 35) displayed the highest genetic distance of one, whereas five other pairs (5 and 38, 12 and 19, 20 and 30, 32 and 37, 46 and 52) revealed zero distance, indicating their exact morphological similarity. But the genetic distance of AFLP supported none of these morphological similarity or dissimilarity, indicating the presence of huge

discrepancy between morphological and AFLP methods. Correlation between the two genetic distances was also very weak, confirming the same result.

Several other comparisons between morphological and molecular-based studies also indicated that these two methods were different and highly variable (Ben-Har *et al.*, 1995; Burstin and Charcosset, 1997; Roldan-Ruiz *et al.*, 2001). As the latter authors emphasized, such observations should not be regarded as indication of a weakness or limitation of these two systems. Genotypes that display high phenotypic similarity need not be genetically similar, as environment plays significant roles in phenotypic expression. Had similarity of molecular and morphological relationship been found, it might have indicated little effect of environment or a very restricted commercial gene pool (Roldan-Ruiz *et al.*, 2001). Consistency should, therefore, be expected if environment has no impact and if the accessions performed identically across the environments, which was not necessarily the case in this study. However, the characterization with morphological and AFLP analyses independently provided the overall interrelationship of the studied accessions. Morphological traits are expected to provide the general representation of the accession relationships according to their growing environment, while the AFLP provides a more accurate and reliable relationships, as it deals with their basic DNA sequences.

UPGMA-based cluster analysis was used to explore the pattern of genetic diversity in the studied accessions of linseed. Cluster that was constructed on the basis of morphological traits was different from that of AFLP (Table 9.5 and Figure 9.3). The former had 18 groups, while the latter consisted of 13 divisions. The constituents of these groups were also different due mainly to the major effects of environment on the 12 quantitative traits used for morphological descriptors. Quantitative traits that are controlled by a number of genes with small effects are tremendously influenced by environment (Poehlman, 1987).

Table 9.5. Cluster distribution of 60 linseed accessions based on morphology (upper) and AFLP (lower) data analysis, 2002

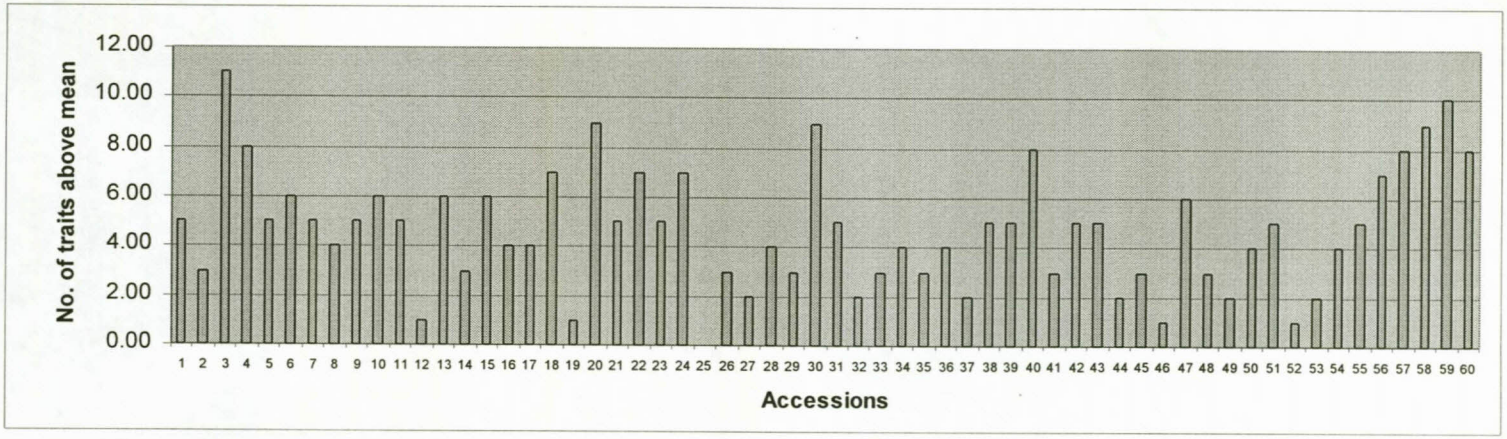
Cluster	No. of acc.	Serial number of accessions and their collection areas, as abbreviated* in the parenthesis
Morphology		
I	3	5 and 16 (H-E), 38 (I-W)
II	2	15 (G-NW), 54 (Eth)
III	7	2 (S-S), 32 and 46 (G-NW), 37 (W-N), 49, 50 and 52 (Eth)
IV	3	13 (G-NW), 17 (H-E), 33 (S-C)
V	5	20 and 30 (Europe), 58 and 59 and 60 (Canada)
VI	3	14 (GG-SW), 23 (S-C), 26 (W-W)
VII	8	9 (Eritrea), 10 (A-S), 24 (S-S), 39 (T-N), 40 (S-C), 42 (B- S), 56 (USA), 47 (G-NW)
VIII	6	1, 21 and 36 (S-C), 6 (W-N), 7 (G-NW), 51 (Eth)
IX	2	3 (K-SW), 4 (G-NW)
X	2	8 (S-S), 31 (Gr-NW)
XI	3	34 and 35 (S-C), 43 (T-N)
XII	2	45 (G-SW), 53 (Eth)
XIII	9	11 (B-S), 12 (W-W), 19 (S-C), 25 (T-N), 27 (W-N), 29 (Gr-SW), 41 (I-W), 44 (Gr-NW), 48 (Eth
XIV	1	18 (S-C)
XV	1	22 (S-C)
XVI	1	28 (G-NW)
XVII	1	55 (Eth)
XVIII	1	57 Canada)
AFLP		
I	2	57 and 58 (Saskatoon/ Canada)
II	5	52, 53, 54 and 55 (Eth), 56 (USA)
III	2	47 and 46 (G-NW)
IV	3	13 and 28 (Gr-NW), 14 (GG-SW)
V	2	32 (G-NW), 34 (S-C
VI	5	7 (G-NW), 18 and 35 (S-C), 30 (Europe), 31 (Gr-NW)
VII	11	15 (G-NW), 16 and 17 (H-E), 19, 22 and 23 (S-C), 24 (S-S), 25 (T-N), 26 (W-W), 27 (W-N), 29 (Gr-NW)
VIII	6	11 (B-S), 12 (W-W), 21, 33 and 51 (S-C), 60 (Canada)
IX	14	1 (S-C), 2 and 8 (S-S), 3 (K-SW), 4 (Gr-NW), 5 (H-E), 6 (W-N), 9 (Eritrea), 10 (A-S), 36 (S-C), 42 (B- S), 48, 49 and 50 (Eth)
X	2	39 (T-N), 41 (I-W)
XI	6	37 (W-N), 38 (I-W), 40 (S-C), 43 (T-N), 44 (Gr-NW), 45 (GG-SW)
XII	1	20 (Europe)
XIII	1	59 (Canada)

* H-E = Hararge-East; I-W = Ilubabor-West; G-NW = Gojam-North West; T-N = Tigray-North; B-S = Bale-South; Sidamo-South; Arsi-South; K-SW = Kefa-South West; S-C = Shewa-Centre; Gr-NW = Gonder-North West; W-N = Welo-North; W-W = Welega-West; GG-SW = Gamo-Gofa-South West; A-SE = Arsi South-East, parts of Ethiopia; Eth = Ethiopia, specific location could not be traced

None of the constructed clusters contained all accessions assembled from a particular collection area, indicating the absence of correspondence between the accessions and their geographical origin. It was evident from the AFLP cluster (Table 9.5) that two introduced accessions (20 and 59) were the most distantly related to the remaining accessions, followed by two accessions each grouped in clusters I, III, V and X. This shows that the range of genetic diversity was greatest among accessions contained in these four clusters and cross hybridising between them may increase genetic variation in the breeding population. The other purpose of clustering would be to subdivide accessions into similar groups so that duplications could be avoided for efficient use of resources during germplasm rejuvenation and preservation.

Generally, morphological clustering of linseeds was different from that of AFLP-based analysis in the present study. Similar results were also reported in the diversity studies of maize inbred lines (Swanepoel, 1999) and perennial ryegrass (Roldan-Ruiz *et al.*, 2001). This reflects the problem of morphological characterization in which it is highly influenced by environment and different growth stages (Kumar, 1999). This implies that the AFLP method could be more important and reliable, as it was reported in many previous studies (Powell *et al.*, 1996; Tohme *et al.*, 1996; Barret and Kidwell, 1998; Swanepoel, 1999). These workers indicated that AFLP markers are highly efficient compared to morphology and other DNA markers, as AFLP markers are reproducible, and display intraspecific homology (Swanepoel, 1999).

A growing number of recent studies have shown the capacity of AFLP to be highly discriminating between genotypes in a range of crops, like wheat (Barret *et al.*, 1998), rice (Mackil *et al.*, 1996), and rapeseed (Lombard *et al.*, 2002). Nevertheless, some studies (Lubberstedt *et al.*, 1998; Swanepoel, 1999; Roldan-Ruiz *et al.*, 2001) suggest that the combination of morphological and molecular markers could serve as a major source of information in separating closely related accessions of different crops. In the current study, AFLP also disclosed a useful genetic variability among the linseed collections though little agreement was noted on the relationship of AFLP and morphological methods. Roldan-Ruiz *et al.* (2001) also found similar inconsistent relationships between morphology and AFLP in the comparative study of molecular and morphological methods of describing



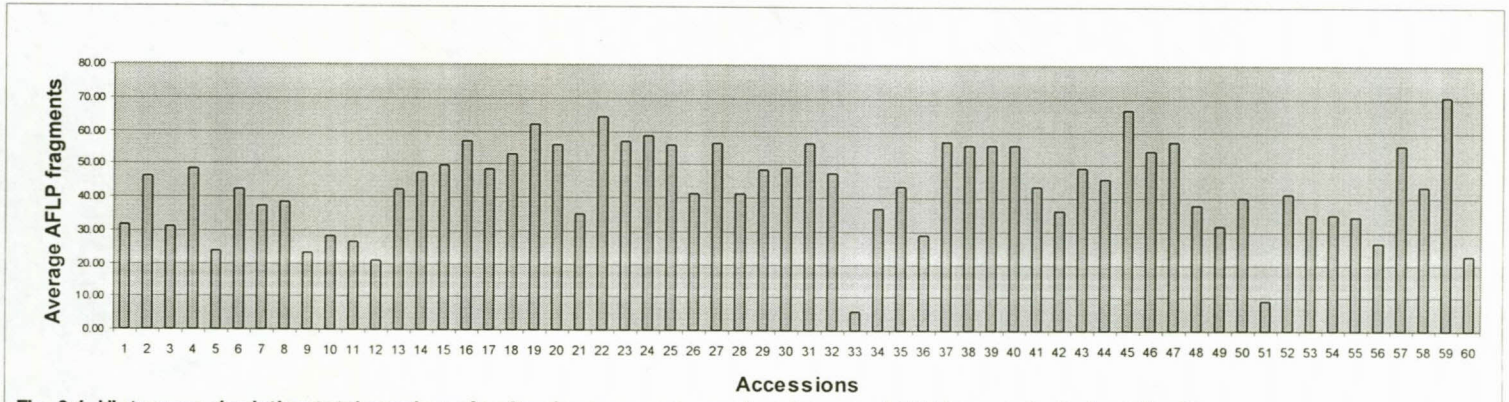


Fig. 9.1. Histogram depicting total number of traits above mean (upper) and averge AFLP fragments (below) for 60 linseed accessions

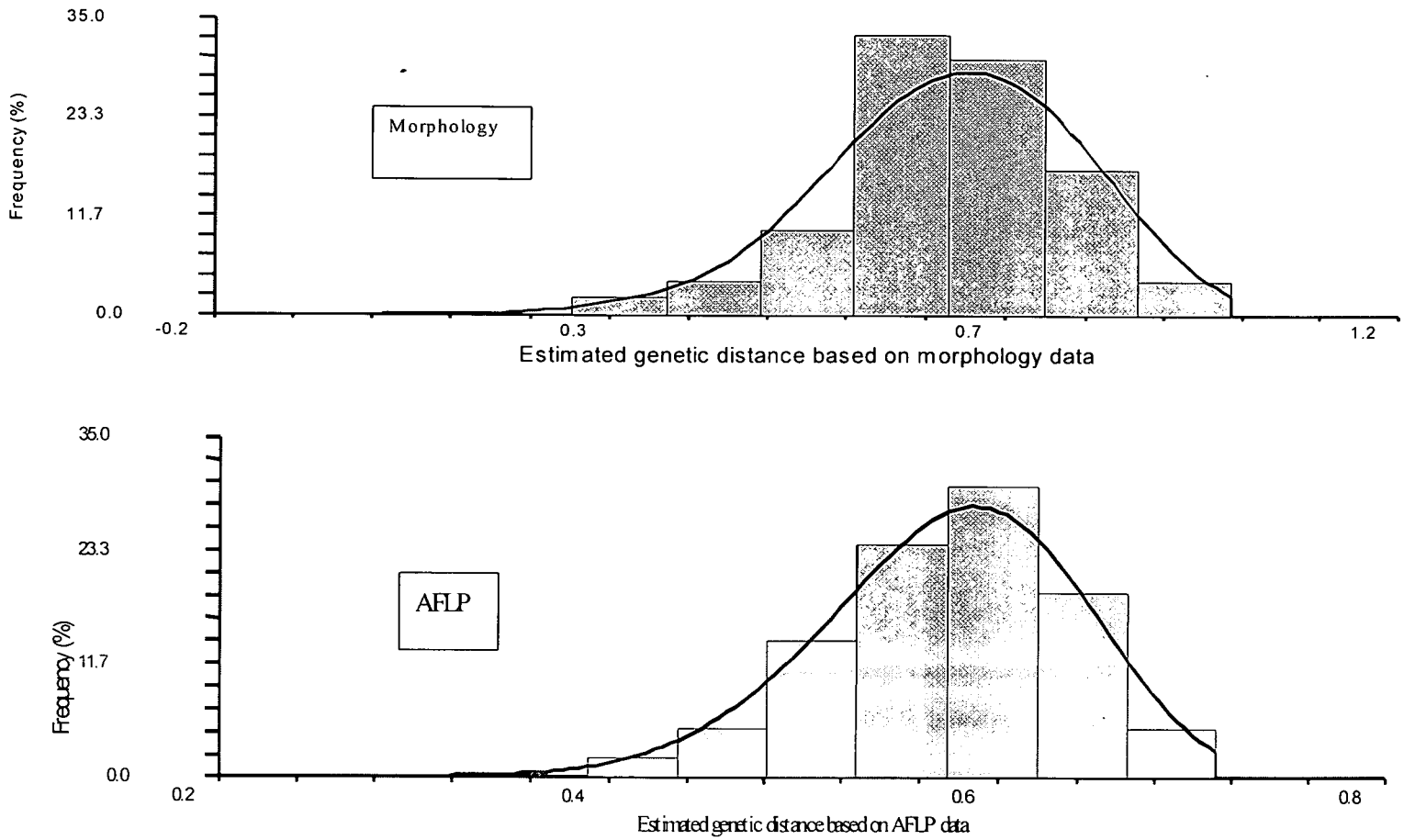


Fig. 9.2. Histogram showing frequency distribution of genetic distances for all pairs of 60 linseed accessions (%) for morphological and AFLP data

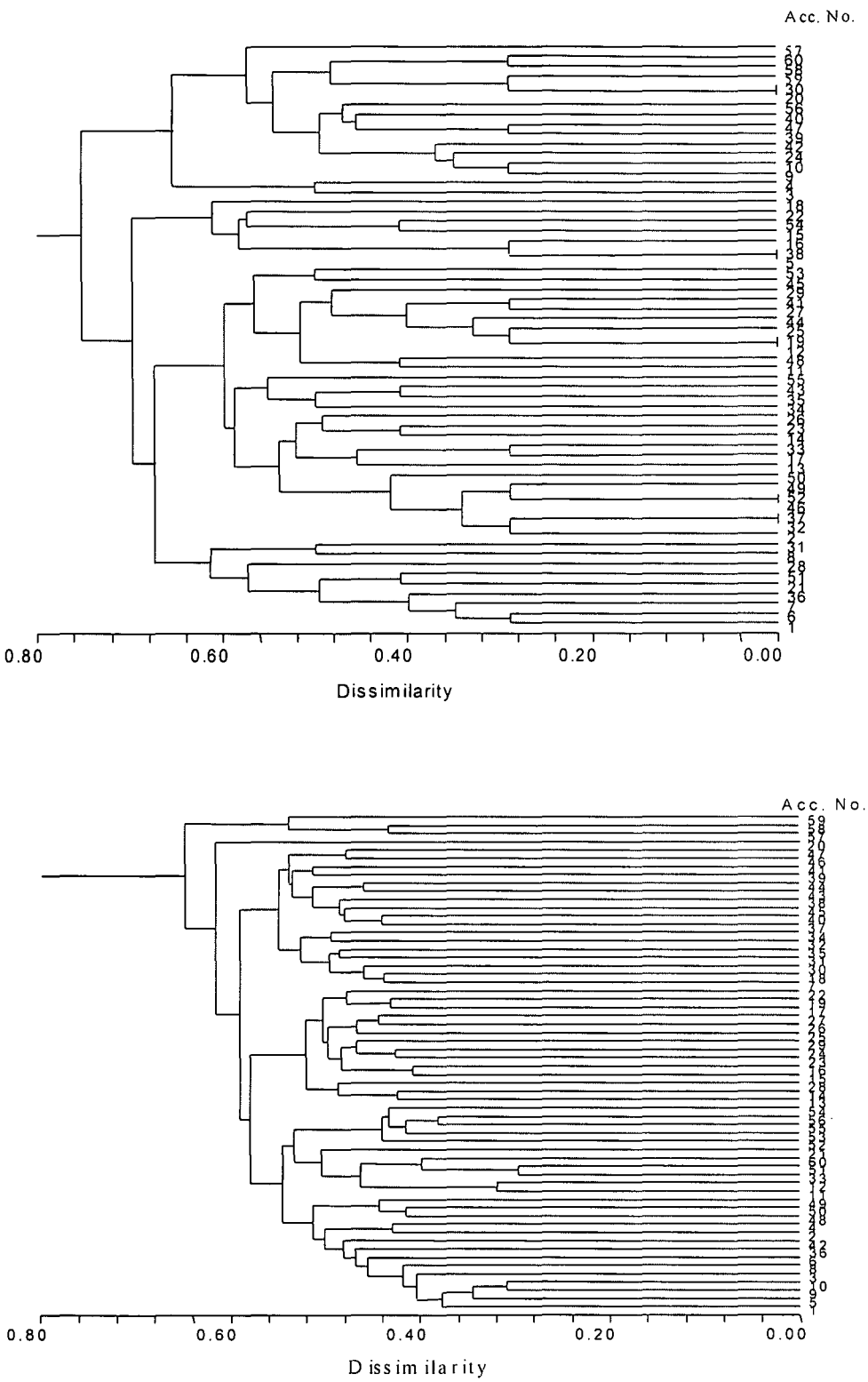


Fig. 9.3. Dendrogram indicating clusters of 60 accessions of linseed based on morphological data (upper) and AFLP data (below), using UPGMA clustering method

relationships among varieties of perennial ryegrass. Ben-Har *et al.* (1995) have also reported a similar disparity between phenotypic and molecular study in maize.

In morphological analysis of the present study, it took almost two years to collect data of 12 quantitative traits that were tested from 2000 to 2002 under glasshouse and field conditions. Similarly, it took about one and a half year to obtain this AFLP results. The ups and downs of laboratory processes were not easy, as various resources were limiting and we were dependent on imported reagents and supplies, which often took long to arrive. However, AFLP seems more efficient than morphology though all their costs and other technical problems were not compared in detail. In this regard, one cannot undermine the roles that have been played by morphological characterization though it has its own limitations. It has been in the service of germplasm classification and identification for the last thousand years. It is still useful in many practical aspects of crop improvement and germplasm maintenance programs, particularly in many developing countries, like Ethiopia. It is still important despite its limitations. Its practical means of making progress in germplasm evaluation schemes in many developing countries and its continuous services are, therefore, beyond doubt for farmers, breeders and germplasm curators for many years to come.

The major set-backs of using molecular markers in developing countries for characterization of germplasm is their initial cost and unsustainability due to inadequate infrastructure, poor maintenance and expensive chemicals and supplies. Although new techniques, such as AFLP markers are more efficient and provide exciting insights (Kumar, 1999), they have their own costs and limitations, especially in developing countries where various resources are scarce. Nevertheless, the values and needs for molecular characterization and MAS were well recognized for genetic resource centres and crop improvement programs despite their high costs. The progress with DNA markers could accelerate the process of finding markers related with specific traits of interest, such as disease and pest tolerance (Spielmeyer *et al.*, 1999). This may offer a powerful set of tools for analysing the inheritance of important traits in linseed breeding. In summary, fair and better comparison of the two methods in terms of feasibility, efficiency and accuracy need to be done by including reasonable number of samples, more multi-environment tests and other

DNA markers (e.g., SSR) along with their costs and benefits, and this should be the focus of future research.

9.6 Conclusions

Morphological and AFLP-based genetic distance and cluster analyses showed little convergence in the current study, as well as in other previous studies, as described in the above discussion. AFLP reflects the true expression of genotype, while morphology encompasses the expression of genotype, environment and their interaction. Thus, the latter remains subjective and inconsistent, whereas the former appeared to provide more accurate estimates and utility of genetic diversity measurements. In short, both methods have their own pros and cons for practical applications under different circumstances; therefore, both will continue contributing their valuable services to farmers, breeders and genetic resource curators for many years to come.

CHAPTER 10

CONCLUDING REMARKS

Science-based information on the levels and patterns of genetic diversity is valuable for efficient management of germplasm and for effective utilization of the materials in the breeding programs. Genetic diversity is always vital to meet the ever-changing needs of growers and consumers in the face of changing and unpredictable environmental challenges. High genetic diversity implies sustainable and stable productivity, buffering various biotic and abiotic stresses.

Based on the above consensus, analyses of genetic diversity were undertaken in 60 linseed accessions under glasshouse and field conditions. The results revealed the presence of wide ranges of genetic diversity among these accessions. The results also showed a rich diversity for 12 quantitative traits, such as earliness and high oil content. This suggests the availability of ample opportunities for genetic improvement through selection and cross breeding. Exotic accessions, particularly those introduced from Canada displayed higher oil contents and other desirable qualities than the local ones. Therefore, the Ethiopian linseed research can benefit more if linkages are strengthened with research and universities working on linseed, such as the Universities of Saskatchewan and North Dakota in North America.

The combined analyses of variance across environments showed tremendous influences of environmental factors in the expression of various characters. Better expression of characters and genetic parameters (e.g., heritability and expected genetic gain) were noted under field conditions than in the glasshouse. Thus, future evaluation and breeding activities need to be undertaken under target and appropriate environments, such as the Holetta area. The integrated analyses of genetic and environmental factors were found to be a better strategy for more effective and efficient germplasm enhancement programs. Multi-environment tests help to obtain a better picture of the accessions and can offer more opportunities of selecting genotypes for broad and/ or specific environments.

The significant variability in oil content, oil yield and fatty acids indicates the possibility of further improvement, giving more emphasis to the exotic accessions, such as the Canadian accessions through continuous germplasm exchange programs. The high level of unsaturated and low saturated fatty acids in the current accessions reveals the fitness of these materials for healthy food products. The identified level of variation of linolenic fatty acid in current accessions was inadequate to develop genotypes with a value lower than 2% for cooking oil quality via conventional breeding. Hence, trying to introduce the already developed ones or using mutation breeding would be the better options.

The strong positive association between yield per unit area and yield/plant, bolls/plant and thousand seed weight is of considerable importance in improving the efficiency of linseed breeding through selection for these characteristics. The strong negative correlation between unsaturated fatty acids and saturated ones indicates the desaturation processes of linseed oil from oleic to linoleic and then to linolenic fatty acids during their biosynthesis. Since quality of linseed oil is dependent on the composition of fatty acids, and they are much influenced by various factors, such as genotypes, environment and other correlated traits, all of these components should be considered to achieve the required oil quality. Better understanding of the interrelationships between the influential factors are necessary in formulating selection criteria and thus to enhance progress of oil quality improvement.

AFLP markers displayed the prevalence of genetic diversity in both exotic and local accessions of linseed. The Ethiopian accessions showed within and between regional variability. AFLP was effective in discriminating the linseed germplasm and in identifying parents for future crossing. Crossing breeding programs should make use of these divergent accessions that were detected by AFLP markers to achieve fast progress. AFLP-based genetic information may increase the efficiency of breeding and germplasm management. Future research has to concentrate on the use of more DNA markers, such as SSR by targeting specific traits, like tolerance to drought, frost, parasitic weeds and *Fusarium* wilt.

Both morphological and AFLP characterization showed different amounts and patterns of genetic diversity among the studied accessions. The level of variation was higher for morphological descriptors than for AFLP markers in terms of the genetic distance and number of constructed clusters. Their correlation was also very weak and thus there was little correspondence between the two methods. AFLP appears to provide more accurate and reliable information but it is expensive and resource demanding. Morphology was subjective and showed continuous variation due to high environmental influences. Generally, both methods have their own advantages and disadvantages, and one cannot substitute the other. Therefore, the results of both methods should be used in supplementing and complementing each other.

In summary, further collections should be strengthened focusing on drought-prone areas, remote localities and regions with higher diversity, such as north, northwest and southeast parts of Ethiopia to capture and retain sufficient gene pools for generations to come. This should be done in collaboration with farmers, the Ministry of Agriculture and other key informants. *Ex situ* conservation appears to be a more realistic, sound and safe strategy than the *in situ* system under the current rapidly changing environmental and socio-economical conditions of Ethiopia. A selective sampling strategy focusing on major constraints (e.g., drought, frost, pests and diseases) in northern, southern, western and central Ethiopia could help in securing valuable gene sources. The identified variation should also be utilized to develop better performing cultivars.

CHAPTER 11

SUMMARY

The study was carried out to assess the genetic diversity of 60 linseed accessions mainly collected from Ethiopia in different environments using morphological and amplified fragment length polymorphism (AFLP) markers from 2000 to 2002. AFLP and morphological characterization were conducted under glasshouse conditions at the University of the Free State in South Africa. Similarly, morphological evaluations were undertaken under field conditions at Holetta Research Centre in Ethiopia during 2000 and 2001 cropping seasons. The main objectives of the study were to determine the levels and patterns of genetic diversity along with other genetic parameters using both morphological descriptors and the AFLP markers, and to compare the usefulness and relationships of these two methods in discriminating the accessions of linseed by applying univariate, bivariate and multivariate statistical analyses.

The analysis of variance for the glasshouse experiment showed highly significant difference ($P < 0.01$) among the accessions for 11 quantitative traits measured, indicating the presence of high genetic diversity. This result was also confirmed by the principal component analysis (PCA) and cluster analysis (CA). PCA displayed that days to flowering, maturity and seeds/boll accounted for 20% of the total variability. CA grouped the accessions into 11 main clusters, consisting one to 24 accessions each. No correspondence was found between clustering and geographic origins of the accessions. Estimation of broad sense heritability and predicted genetic gains (as percent of mean) were also computed to forecast the possible genetic advance in the future. Heritability ranged from 15.60% for seed yield/plant to 85.82% for initial days to flowering, whereas predicted genetic gains varied from 3.16% for days to maturity to 24.26% for plant height.

The analysis of variance for the field experiments revealed highly significant differences ($P < 0.01$) among the accessions for 11 characters. PCA and CA also denoted the same thing, showing the presence of a wide range of genetic variations

between the accessions studied. CA clustered the accessions into nine classes, each consisting one to 39 accessions. The clustering was independent of collection areas. Phenotypic coefficient of variation ranged from 6.26% (days to maturity) to 54.97% (seed yield/plant), while genotypic coefficient of variation differed from 5.46% to 50.18% for the same characters. Heritability (broad sense) was in the range of 67.38% to 91.38%, whereas the predicted genetic gain varied from 9.38% to 94.37%. In both cases, the highest value was estimated for seeds/boll, while the lowest was for days to maturity.

A combined analysis of variance of 10 quantative traits across four environments (two localities and two years) displayed highly significant differences ($P < 0.01$) among the accessions for all characters. There was also significant difference between the localities, years and their interactions for most characters, indicating the differential responses of the accessions across environments. PCA and CA also confirmed the differences between accessions and the prevalence of diversity among the accessions. PCA displayed that secondary branches/plant, plant height, days to flowering and seed yield/plant played major roles in differentiating the accessions. CA grouped the accessions into nine major clusters based on their mean performance rather than their geographic origins. Broad sense heritability and expected genetic advance were found higher across multi-environments than for the single one, indicating the importance of evaluating germplasm under different and appropriate environments. Both parameters were highest for plant height and days to flowering, indicating the effectiveness of selection for these traits.

Diversity analyses for oil content, oil yield and fatty acid profiles generally indicated significant ($P < 0.01$) variations among the studied accessions. Oil content varied from about 29 to 36%, while oil yield ranged between 1443 and 3276 g/m^2. The highest oil yield was obtained from Belay-96, followed by CDC-VG (3212 g/m^2), an introduction from Canada, which was identified as one the most promising genotypes in this study. Fatty acids that are principally grouped into two main groups (saturated and unsaturated) showed wide ranges of variation. Saturated fatty acids (palmitic and stearic) ranged from about 8 to 12%, whereas

the unsaturated (oleic, linoleic and linolenic) ones significantly ($P < 0.01$) varied from 85 to 91%. The highest variation was recorded for oleic acid (14-29%), followed by linolenic (47-59%) and linoleic (10-16%) fatty acids. The accessions were grouped into six cluster based on their oil content, oil yield and linolenic acid, but into 10 classes based on all their fatty acid profiles.

Associations of characters were analysed across different environments to determine the extent and consistency of correlations and to identify major yield attributes of linseed. The degrees of correlation varied across years and locations due mainly to climatic factors, such as temperature, moisture regime and disease incidences. Seed yield was significantly ($P < 0.01$) and positively correlated with yield/plant, bolls/plant and 1000-seed weight. These three traits were also strongly and positively related with plant height, branches/plant and days to maturity. Oil yield was significantly and positively associated with polyunsaturated (linoleic and linolenic) fatty acids, while it was negatively correlated with saturated (palmitic and stearic) fatty acids. The results generally showed tremendous variations in correlations of characters in response to the growing environments.

The current accessions of linseed were assessed for their genetic variation by using AFLP markers. The estimated genetic distance for the entire pairwise accessions varied from 0.29 to 0.71. The top three pairs of accessions with the highest genetic distance were 22 and 57, 25 and 57 and 20 and 45. High levels of polymorphism were also observed for the analysed accessions, indicating the prevalence of genetic diversity in both exotic and local collections. Collections from central and northwest regions of Ethiopia revealed considerable variations, implying further explorations in these areas. Cluster analysis grouped the accessions into 13 classes, each consisting one to 14 accessions. AFLP was found effective in discriminating and grouping the accessions for more predictable breeding and efficient management of genetic resource in the future.

Combining phenotypic and genotypic assessment of genetic diversity could help in acquiring more reliable genetic information for discriminating germplasm and cultivars. To this end, morphological and AFLP data were employed to investigate

the levels and patterns of variation existing in linseed accessions and to compare the two methods. Analyses of ANOVA, PCA and CA revealed the existence of wide range of genetic diversity among the accessions. The average genetic distance of all pairwise accessions was 0.6684 for morphology and 0.5734 for AFLP markers. Higher level of variation was noted for morphology than for AFLP. Correlation between the two distances was very weak and their clustering patterns were also different. In fact, clustering patterns were variable based number of traits and environments tested. In spite of this disparity, however, both methods were found independently adequate and useful in germplasm characterization and/ or cultivar identification based on circumstances, like the availability of research facilities, fund and other essential resources.

OPSOMMING

Hierdie studie is uitgevoer om die genetiese diversiteit te bepaal van 60 lynsaad genotipes wat hoofsaaklik uit Etiopië afkomstig is, met die gebruik van morfologiese en AFLP (amplified fragment length polymorphism) merkers in die tydperk 2000 tot 2002. AFLP en morfologiese karakterisering is gedoen onder glashuis toestande by die Universiteit van die Vrystaat. Morfologiese evaluasies is ook gedoen onder veld toestande by die Holetta Navorsingsstasie in Etiopië in die 2000 en 2001 groeiseisoene. Die hoof doelwitte van die studie was die bepaling van vlakke en patrone van genetiese diversiteit en die gebruik van ander genetiese parameters wat beide morfologiese eienskappe en AFLP merkers insluit, en om hierdie metodes te vergelyk vir bruikbaarheid en die onderskeiodings vermoë binne die lynsaad genotipes deur die gebruik van monovariate en multivariate statistiese analise.

Variansie analise van die glashuis eksperiment het hoogs betekenisvolle verskille ($P<0.01$) tussen inskrywings aangetoon vir die 11 kwantitatiewe eienskappe gemeet, wat 'n aanduiding gee van hoë genetiese diversiteit. Die resultate is bevestig met PCA (principle component) analise en CA (cluster analysis). PCA het aangedui dat dae tot blom, rypwording en sade per bol 20% van variabiliteit verklaar het. CA het die inskrywings in 11 hoof groepe ingedeel, wat uit een tot 24 inskrywings bestaan het. Geen ooreenkoms is gevind tussen groeperings en geografiese oorsprong van inskrywings nie. Die bepaling van breë sin oorerflikheid en voorspelde genetiese vordering (as persent van die gemiddeld) is gedoen om moontlike genetiese vooruitgang te voorspel. Oorerflikheid het gewissel van 15.6% vir saad opbrengs per plant tot 85.82% vir dae tot blom. Voorspelde genetiese vordering het gevarieër van 3.16% vir dae tot ryp tot 24.6% vir plant hoogte.

Variansie analise vir die veld proef het betekenisvolle ($p<0.01$) verskille aangetoon tussen inskrywings vir die 11 eienskappe. PCA en CA het dieselfde aangetoon, wat 'n wye reeks van genetiese variasie aandui vir die inskrywings. CA het die inskrywings in nege groepe gedeel wat een tot 39 inskrywings gehad het. Die groeperings was onafhanklik van versamelings plekke. Fenotipiese koeffisiënt van

variasie het gewissel van 6.26% (dae tot ryp) tot 54.97% (saad opbrengs per plant). Genotipiese CV het gewissel van 5.46% tot 50.18% vir dieselfde eienskappe. Oorerflikheid (breë sin) het gewissel van 67.38% tot 91.38%, en die verwagte genetiese vordering het gewissel van 9.38% tot 94.37%. In beide gevalle was die hoogste waarde vir saad per bol, en die laagste vir dae tot rypheid.

'n Gekombineerde variansie analise vir 10 kwantitatiewe eienskappe oor vier omgewings (twee lokaliteite en twee jare) het betekenisvolle verskille ($P<0.01$) tussen inskrywings vir alle karakters getoon. Daar was ook betekenisvolle verskille tussen lokaliteite, jare en hulle interaksie vir meeste eienskappe, wat differensiële response van inskrywings oor omgewings aantoon. PCA en CA het ook die verskille tussen inskrywings beklemtoon, en die teenwoordigheid van diversiteit tussen inskrywings. PCA het gewys dat sekondêre vertakkings per plant, plant hoogte, dae tot blom en saad opbrengs per plant 'n groot rol speel in die differensiasie van die inskrywings. CA het inskrywings in nege groepe verdeel gebaseer op gemiddelde prestasie eerder as geografiese oorsprong. . Breë sin oorerflikheid en verwagte genetiese vordering was hoër oor omgewings as in enkele omgewings, wat die belangrikheid onderstreep om kiemplasma te evalueer onder verskillende en geskikte omgewings. Beide hierdie parameters was die hoogste vir plant hoogte en dae tot blom wat aandui dat hierdie eienskappe effektief geselekteer kan word.

Diversiteits analise vir olie inhoud, olie opbrengs en vetsuur profiele het oor die algemeen betekenisvolle ($P<0.01$) variasies tussen inskrywings getoon. Olie inhoud het gewissel tussen 29% tot 36%, en olie opbrengs tussen 1443 tot 3276 g/m^2. Die hoogste olie opbrengs is gekry van Belay-96, gevolg deur CDC-VG ($3212g/m^2$), 'n introduksie van Kanada, wat identifiseer is as een van die mees belowende genotipes in hierdie studie. Vetsure wat gegroepeer is in twee hoof groepe (versadig en onversadig) het groot variasie getoon. Versadigde vetsure (palmities en stearien suur) het gewissel van 8% tot 12%, en onversadigde (oleïen, linoïen en linoleen) vetsure het betekenisvol gevarieër van 85% tot 91%. Die hoogste variasie is aangetoon vir oleïensuur (14-29%), gevolg deur linoleensuur (47-59%) en linoïen (10-16%) vetsure. Die inskrywings is gedeel in ses groepe

gebaseer op olie inhoud, olie opbrengs en linoleensuur, maar in 10 klasse gebaseer op vetsuur profiele. .

Assosiasie van karakters is geanaliseer oor verskillende omgewings om die omvang en herhaalbaarhied van korrelasies vas te stel, en om die grootste bydraers van opbrengs by lynsaad te bepaal. Die graad van korrelasie het gewissel oor jare en lokaliteite a.g.v. klimatiese faktore soos temperatuur, vog en siekte insidensie. Saad opbrengs was betekenisvol ($P<0.01$) en positief gekorreleer met opbrengs per plant, kapsules per plant en 1000 saad gewig. Hierdie drie eienskappe was ook sterk positief gekorreleer met plant hoogte, vertakkings per plant en dae tot rypheid. Olie opbrengs was betekenisvol en positief gekorreleer met poli-onversadigde (linoïen en linoleen) vetsure, terwyl dit negatief gekorreleer is met versadigde (palmitiese en stearien) vetsure. Die resultate het oor die algemeen geweldige variasie gewys in korrelasies van karakters in reaksie op omgewings toestande.

Met die gebruik van AFLP is die lynsaad inskrywings geëvaleer vir genetiese variasie. Die genetiese afstand vir paarsgewyse vergelykings het gewissel van 0.29 tot 0.71. Die top drie paar inskrywings met die grootste genetiese afstande was 22 en 57, 25 en 57 en 20 en 45. 'n Hoë vlak van polimorfisme is waargeneem vir die geanaliseerde inskrywings wat heelwat genetiese diversiteit aantoon in beide eksotiese en plaaslike versamelings. Versamelings van sentraal en die noordwestelike areas van Etiopië het aansienlike variasie getoon, dus moet hierdie gebiede verder ondersoek word. Groep analise het die inskrywings in 13 klasse gedeel, met elk een tot 14 inskrywings. AFLP's was effektief om inskrywings te onderskei en te groepeer vir meer effektiewe teling en beter bestuur van genetiese bronne in die toekoms.

Gekombineerde fenotipiese en genotipiese assessering van genetiese diversiteit kan help om meer betroubare genetiese inligting te kry om kiemplasma en cultivars te onderskei. Om dit te doen is morfologiese en AFLP data gebruik om die vlakke en patrone van variasie in lynsaad genotipes te bepaal en om die twee metodes te vergelyk. ANOVA, PCA en CA analises het wye genetiese diversiteit aangetoon in

inskrywings. Die gemiddelde genetiese afstand vir alle paarsgewyse vergelykings was 0.6684 vir morfologiese en 0.5734 vir AFLP merkers. 'n Hoër vlak van variasie was teenwoordig vir morfologie as vir AFLP. Korrelasie tussen die twee afstande was swak, en groeperings patrone was verskillend. Ten spyte van verskille, is beide metodes effektief gevind om afsonderlik gebruik te word vir kiemplasma karakterisering en/of cultivar identifikasie afhangend van omstandighede soos die beskikbaarheid van navorsings geriewe, fondse en ander belangrike hulpbronne.

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APPENDICES

Appendix 1. Reagents and methods used for oil extraction (Folch et al., 1957) and methylation (Slover and Lanza, 1979) of linseed accessions at UFS, South Africa, 2001

OIL EXTRACTION

Reagents:

A/ Upper phase: Chloroform:methanol (2: 1 v/v) with 0.001 % BHT

1. Put 0.02 g 20 mg BHT in 2000 ml volumetric flask.
2. Add 1333.33 ml chloroform.
3. Add 666.67 ml methanol.
4. Shake thoroughly.
5. Mark flask clearly.

B/ Lower phase: Choroform:methanol:water (86:14:1 v/v)

1. Put 1702.97 ml chloroform in 2000 ml volumetric flask.
2. Add 277.23 ml methanol.
3. Add 19.8 ml water.
4. Shake thoroughly.
5. Mark flask clearly.

Method:

1. Weigh ± 0.5 g ground seed in 250 ml clearly marked round bottom flask.
2. Annotate weight accurately.
3. Add 30 ml of 2:1.
4. Put contents under a blanket of nitrogen and put lid on flask.
5. Leave overnight in refrigerator at 4 °C.
6. Weigh clearly marked 18.5 cm Whatman nr. 1 filter paper accurate to the fourth decimal, fold and put in funnel on top of separating funnel.
7. Measure 37 ml of 2:1 in 100 ml measuring cylinder.
8. Transfer sample in round bottom flask to filter paper on top of funnel.

9. Wash round bottom flask three times with 12.33 ml 2:1 and transfer each time to filter paper.
10. Allow everything to run through filter paper and remove funnel with filter paper.
11. Add 16.5 ml water to each separating funnel.
12. Cap separating funnels, shake thoroughly, and allow one hour for separation.
13. After 1 hour drain lower phase into 500 ml round bottom flask.
14. Add 50 ml lower phase to each separating funnel.
15. Cap separating funnels, shake thoroughly, and allow 15 minutes for separation.
16. After 15 minutes drain lower phase into the same 500 ml round bottom flask, repeat steps 14 to 16.
17. Discard upper phase.
18. Evaporate contents of 500 ml round bottom flask under vacuum in rotary evaporator at 60 °C for 20 minutes at 15 psi.
19. Remove remaining water by the addition of 45 ml of methanol and remove it by evaporating each round flask at 60 °C at 15 psi.
20. Weigh clearly marked polytop with lid accurately to four decimals and annotate.
21. Wash contents of each 500 ml round bottom flask with 6 x 5 ml portions of diethyl ether into preweighed polytop.
22. Remove diethyl ether from each polytop by evaporation under a steam of nitrogen on heating block at 60 °C for 20 minutes.
23. Put filter papers and polytops in vacuum over and dry at 50 °C overnight.
24. Remove next morning and weigh filter papers and capped polytops.
25. Calculate: % Fat, Fat Free Dry Matter, and % moisture.
26. Transfer ± 10 mg (will later be used for methylation) to another polytop and annotate weight of sample.

METHYLATION

Reagents: Hexane, BF_3-Methanol (14%), Saturated NaCl (45 g NaCl + 105 ml H_2O

Na_2SO_4, 0.5 N NaOH in methanol 2 g of NaOH is dissolved in 100 ml of methanol containing not more than 0.5% of water.

Method:

1. An amount of ± 10 ml of oil are put or washed over with 6 x 1 ml hexane into the rest tube with Teflon-lined cap.
2. Remove hexane by evaporation without application of heat.
3. Add 1 ml 0.5 N methanolic NaOH to the test tube, cap and heat in a boiling water bath for 15 minutes.
4. After cooling of the test tube, add 2 ml of BF3-Mehanol (14%).
5. Recap and heat in boiling water bath for additional 15 minutes.
6. Cool the test tube and add 1 ml of hexane and 2 ml of saturated NaCl and shake vigorously for 1 minute.
7. Keep and allow the phases to separate.
8. Transfer 70% of the upper hexane layer to an autosampler vial containing a 1 ml Na_2SO_4, layer, close and allow to stand for 20 minutes to remove traces of water.
9. Transfer 100 µl hexane from each vial to a clean labelled autosampler vial.
10. Add 900 µl hexane to each authosampler vial.
11. Store below freezing point before GC analyses.

Appendix 2. Genomic DNA extraction procedure

1. Gather 0.5-2gm fresh plant material
2. Preheat the extraction buffer to 65 °C
3. Grind plant material with mortar and pestle in liquid nitrogen.
4. Scoop pulped material into labelled, clean centrifuge tube.
5. Add 10ml extraction buffer, 2ml 5M NaCl and 1ml CTAB.
6. Incubate at 65°C for 60 minutes, shaking every 15 minutes.
7. Add 10ml (equal volume) chloroform:iso-amylalcohol (24:1).
8. Balance tubes containing plant material for centrifugation.
9. Centrifuge for 15m in at 10 000rpm.
10. Remove top fluid (supernatant) and transfer to new labelled centrifuge tube.

11. Add chilled 99.9% absolute ethanol in 2:1 ratio to transferred fluid.
12. Seal tube with parafilm, and invert gently.
13. Place in fridge and allow DNA to precipitate overnight or for 24 hours.

Cleaning and dissolving of genomic DNA

1. Scoop out DNA-pellet with flamed hockey-stick (Pasteur-pipette)
2. Wash DNA-pellet with 70% ethanol. Repeat 2 times.
3. Transfer DNA-pellet to clean eppendorf tube, and dissolve in 250µl sterile H_2O.
4. DNA concentration can be determined at this point using a spectrophotometer.

Visualisation of genomic DNA on agarose gel

1. Prepare a 1% agarose gel and place in 0.5 x TAE running buffer.
2. Prepare DNA sample by adding to 200ng DNA to 3µl 6x loading buffer.
3. Load samples on gel, and run gel for 45 minutes at 80V.

DNA concentration determination

1. DNA concentration is determined using a spectrophotometer
2. Wash the cuvet using 0.25 M HCl, and rinse with sterile H_2O.
3. Dilute 20 µl of sample DNA in 480 µl sterile H_2O.
4. Zero the spectrophotometer using a cuvet filled with 500 µl sterile H_2O.
5. Take readings at 260nm and 280nm. (DNA absorbs light at this frequency).
6. Purity of the DNA samples is determined using the equation below. DNA purity should be between 1.8 and 2 for best results.
7. DNA concentration is determined using the equation below.

$$\text{Purity} = \frac{\text{260nm reading}}{\text{280nm reading}}$$

$$[\text{DNA}] = \text{optical density x dilution x constant} = \mu g/ml$$

$$= \text{260nm reading x 500/20 x 50}\mu g/ml = \mu g/ml$$

Buffers and solutions

1. Extraction buffer

Solution	For 1l extraction buffer	Final concentration
5 M NaCl	100 ml	500 mM
1 M Tris-HCl pH 8	100 ml	100 mM
0.25 M EDTA pH 8	200 ml	50 mM
20% SDS	62.5 ml	1.25%
DdH_2O	537.5 ml	
TOTAL	1000 ml	

2. Chloroform:iso-amylalcohol

Chloroform	240ml
Iso-amylalcohol	10 ml
TOTAL	250 ml

3. 50 x TAE (stock solution)

Tris	48.44g
0.5 M EDTA	2.92 g
Acetic Acid	11.42 ml
TOTAL	200 ml

4. 0.5 x TAE

50 x TAE	10 ml
ddH_2O	990 ml
TOTAL	1000 ml

5. Agarose gel

0.5 x TAE	50 ml
Agarose	0.5 g

1. Heat until agarose is fully dissolved.
2. Cool slightly.

3. Add 0.5µl Ethidium Bromide. WARNING! Ethidium bromide is carcinogenic and therefore dangerous to your health.
4. Pour into casting tray and allow to set.
5. Transfer to running buffer when set. Helps prevent dehydration of gel.

6. DNA loading buffer

Bromophenol blue	250 mg
Xylene cyanol	250 mg
150mM Tris pH 7.6	33 ml
Glycerol	60 ml
Sterile H_2O	7 ml
TOTAL	100 ml

Appendix 3. Restriction endonuclease digestion, ligation of adaptors and polymerase chain reaction of AFLP markers

Protocol 1: Single digest

A. *Eco*RI:

1.	DNA (conc. 250 ng)	x µl
2.	10 x buffer No.3	1 µl
3.	EcoR I	0.2 µl
4.	Sabax water	10 - $\sum$ (#1-3) µl
	Total	10 µl

B. *Mse*I:

1.	DNA (conc. 250 ng)	x µl
2.	10 x buffer No.2	1 µl
3.	Mse I	0.5 µl
4.	BSA	0.1 µl
5.	Sabax water	10 - $\sum$ (#1-4) µl
	Total	10 µl

1. Add 1µl RNAse to 25 µl DNA dilution and keep in 42°C cabinet for 30 minutes
2. Prepare the above samples by adding DNA, ddH_2O, 10x buffers and enzymes
3. Stir and keep the mixture of DNA for 3-4 hours in the 37°C incubation cabinet.
4. Inactivate the samples by keeping in the fridge overnight.
5. Prepare agarose gel (0.5gm agarose + 50ml 0.5x TAE + 0.5 µl Ethidium bromide)
6. Prepare the 10 µl DNA samples by adding 3 µl 6x loading buffer
7. Load the DNA samples and run for about an hour at 80 volts, using the:
   - first lane for 5 µl Marker III
   - 2-12 lanes for 13 µl DNA samples

Protocol 2: Double digest (DD)

1. 5x reaction buffer	5 µl
2. DNA sample 250ng/µl	< 18 µl = (2.5µl)
3. *Eco*RI/ *Mse*I	2 µl
4. AFLP grade H2O	25 - (# 1 + 3) = (15.5µl)
	25 µl

1. Mix gently and collect the reaction by brief centrifugation.
2. Incubate for 2 hrs at 37 °C in the cabinet.
3. Inactivate the mixture for 15 min. at 70 °C in water-bath or PCR machine. Alternatively, can be placed directly in the fridge without inactivating.
4. Place tubes on ice and collect contents by brief centrifugation.

Protocol 3: Ligation of adapter

DD DNA	25µl
Ligation solution	24 µl
T4 DNA ligase	1 µl
	50 µl

Mix gently at room temp., centrifuge and incubate at 20 °C ± 2 °C for 2 hrs.

Perform 1: 10 dilution of ligation mixture as follows:

a) 10 µl reaction mixture

b) Add 90 µl of TE and mix well (If kits of TE are finished, make your own using sabax water. Unused portion of reaction mixture may be stored at -20 °C).

Protocol 4: Preamlification reaction (PS)

Diluted template DNA	5 µl
Pre-amp. primer mix.	40 µl
10xPCR buffer for AFLP	5 µl
Taq pol. (1 unit/µl)	1 µl (5 units/µl Taq plo.; 0.2 Taq + 0.8 H_2O = 1 µl)
	51 µl

Mix gently and centrifuge. (Remember hot start)

Perform 20 cycles at: 94 °C → 30 sec., 56 °C → 60 sec., and 72 °C → 2 min.

Suck temp. 4 °C.

Run 12 µl on 1% Agarose gel at 80-90 volts with loading buffer = 4 µl

AFTER running Agarose gel, expect to see smear with bands. Bands are highly repeated segments because of mRNA histone, etc.

Perform a 1: 50 dilution as follows:

3 µl rxn + 147 µl TE Dilution sufficient for 30 select. AFLP amp.

Both diluted + undiluted reactions (rxns) can be stored at -20 °C.

Protocol 5: Selective AFLP amplification (Sel)

For each primer pair, add the following components to micro-centrifuge tubes:

Mix 1:

Labeled *Eco*RI primer	10 µl
*MSe*I primer	45 µl
Total volume	55 µl (enough for 10 rxns)

- 5.5 µl can mix in dopre v. eppendorf

Mix 2:

AFLP H2O	
10xPCR pol. (1unit/µl)	
Total volume	100 µl (for 10 rxns)

- 9.5 µl can mix2 on oncler in eppendorf

Reactions

Diluted template DNA	5 µl
Mix 1	5.5 µl
Mix 2	9.5 µl
Total volume	20 µl

Mix gently and centrifuge briefly to collect rxns.

Perform a one cycle at 94 °C → 30 sec; 65 °C → 30 sec; 72 °C → 2 min.

Lower the annealing temp. each cycle 0.7 °C during 12 cycles.

Perform 23 cycles 94 °C → 30 sec.; 56 °C → 30 sec.; 72 °C → 2 min.

Sequencer

Rox	1 µl
Formamide (deionized)	24 µl
DNA product (Ned+Fam)	5 µl
Total	30 µl

Denature DNA at 94 °C for 5 min.; Cool down in ice slowly, twice.

Spin, down take to sequencer; Sucking = 12; Run = 30; Filter set.

Appendix 4. Genetic distances (in descending order) between all possible pairs of accessions based on AFLP data analysis

No.	Acc. no.		Distance	Acc. no.		Distance	Acc. no		Distance	Acc. no.		Distance	Acc. no.		Distance
1	22	57	0.7112	4	57	0.6726	10	57	0.6563	20	59	0.6473	25	31	0.6382
2	25	57	0.7065	11	20	0.6726	14	58	0.6563	22	32	0.6473	38	60	0.6382
3	20	45	0.7018	15	59	0.6726	16	58	0.6563	27	52	0.6473	4	53	0.6369
4	23	59	0.7018	16	59	0.6726	20	46	0.6563	2	21	0.6460	12	39	0.6369
5	37	57	0.7018	21	45	0.6714	44	58	0.6563	16	55	0.6460	15	45	0.6369
6	45	57	0.7018	27	57	0.6714	47	54	0.6563	19	31	0.6460	18	21	0.6369
7	12	59	0.6994	41	59	0.6714	9	57	0.6550	20	30	0.6460	21	58	0.6369
8	23	57	0.6958	19	53	0.6701	20	41	0.6550	21	24	0.6460	22	44	0.6369
9	25	59	0.6958	3	59	0.6689	24	34	0.6550	21	37	0.6460	27	47	0.6369
10	38	59	0.6958	15	57	0.6689	33	45	0.6550	24	54	0.6460	32	59	0.6369
11	24	57	0.6946	29	57	0.6689	40	53	0.6550	37	56	0.6460	38	55	0.6369
12	4	59	0.6934	4	20	0.6676	11	38	0.6537	59	60	0.6460	39	55	0.6369
13	11	59	0.6934	21	57	0.6676	12	20	0.6537	7	57	0.6447	41	57	0.6369
14	22	59	0.6934	27	59	0.6676	18	58	0.6537	19	32	0.6447	41	58	0.6369
15	38	57	0.6922	40	57	0.6676	19	51	0.6537	23	31	0.6447	42	58	0.6369
16	21	59	0.6910	47	53	0.6676	19	60	0.6537	25	32	0.6447	43	58	0.6369
17	24	59	0.6910	13	57	0.6664	22	31	0.6537	39	57	0.6447	3	45	0.6356
18	45	56	0.6910	22	56	0.6664	22	51	0.6537	46	56	0.6447	6	32	0.6356
19	45	53	0.6898	22	60	0.6664	36	57	0.6537	53	59	0.6447	11	39	0.6356
20	45	58	0.6898	23	53	0.6664	39	54	0.6537	5	57	0.6434	16	34	0.6356
21	19	59	0.6886	24	31	0.6664	39	59	0.6537	6	20	0.6434	21	31	0.6356
22	45	59	0.6886	37	59	0.6664	45	52	0.6537	7	59	0.6434	23	55	0.6356
23	1	59	0.6874	45	54	0.6664	46	53	0.6537	21	39	0.6434	24	51	0.6356
24	19	57	0.6874	13	59	0.6651	46	57	0.6537	21	40	0.6434	25	54	0.6356
25	22	58	0.6874	24	53	0.6651	2	20	0.6525	21	47	0.6434	29	58	0.6356
26	9	59	0.6861	28	57	0.6651	2	57	0.6525	22	41	0.6434	40	51	0.6356
27	14	57	0.6861	42	57	0.6651	4	32	0.6525	29	53	0.6434	40	60	0.6356
28	42	59	0.6861	46	59	0.6651	20	40	0.6525	34	57	0.6434	46	58	0.6356
29	10	59	0.6849	20	57	0.6639	23	54	0.6525	40	54	0.6434	1	45	0.6343
30	33	59	0.6849	23	32	0.6639	27	37	0.6525	52	59	0.6434	12	31	0.6343
31	36	59	0.6849	22	53	0.6626	27	54	0.6525	54	59	0.6434	12	40	0.6343
32	14	59	0.6825	37	53	0.6626	39	53	0.6525	16	54	0.6421	13	47	0.6343
33	25	58	0.6825	16	53	0.6614	40	58	0.6525	19	33	0.6421	19	34	0.6343
34	45	51	0.6813	27	53	0.6614	46	54	0.6525	20	24	0.6421	20	31	0.6343
35	45	60	0.6813	6	59	0.6601	4	21	0.6512	20	37	0.6421	23	51	0.6343
36	12	45	0.6800	12	38	0.6601	6	57	0.6512	27	60	0.6421	25	53	0.6343
37	17	59	0.6800	18	59	0.6601	16	20	0.6512	40	55	0.6421	29	54	0.6343
38	37	58	0.6800	20	38	0.6601	20	29	0.6512	50	57	0.6421	29	56	0.6343
39	45	55	0.6800	20	39	0.6601	20	58	0.6512	11	31	0.6408	12	57	0.6330
40	47	57	0.6800	23	56	0.6601	24	56	0.6512	17	58	0.6408	15	20	0.6330
41	11	45	0.6788	26	59	0.6601	25	56	0.6512	27	32	0.6408	15	58	0.6330
42	47	59	0.6788	27	45	0.6601	29	32	0.6512	37	51	0.6408	20	27	0.6330
43	5	59	0.6776	38	53	0.6601	38	54	0.6512	40	56	0.6408	24	52	0.6330
44	19	58	0.6776	1	57	0.6588	38	56	0.6512	47	60	0.6408	37	54	0.6330
45	24	32	0.6776	11	37	0.6588	40	59	0.6512	6	38	0.6395	38	51	0.6330
46	24	58	0.6776	16	32	0.6588	43	59	0.6512	13	45	0.6395	48	57	0.6330
47	34	59	0.6776	22	54	0.6588	4	58	0.6499	24	35	0.6395	11	57	0.6317
48	38	58	0.6776	27	58	0.6588	12	24	0.6499	25	34	0.6395	16	45	0.6317
49	16	57	0.6763	30	57	0.6588	22	33	0.6499	26	45	0.6395	16	60	0.6317
50	23	58	0.6763	47	56	0.6588	22	55	0.6499	36	45	0.6395	20	21	0.6317
51	51	59	0.6763	50	59	0.6588	24	55	0.6499	37	55	0.6395	20	22	0.6317
52	8	59	0.6751	12	37	0.6576	27	31	0.6499	46	52	0.6395	23	60	0.6317
53	29	59	0.6751	16	56	0.6576	30	59	0.6499	48	59	0.6395	24	33	0.6317
54	44	59	0.6751	19	55	0.6576	19	54	0.6486	1	58	0.6382	24	48	0.6317
55	8	57	0.6739	20	47	0.6576	24	47	0.6486	8	58	0.6382	27	40	0.6317
56	17	57	0.6739	27	56	0.6576	26	57	0.6486	10	27	0.6382	39	58	0.6317
57	18	57	0.6739	43	57	0.6576	27	55	0.6486	11	40	0.6382	43	53	0.6317
58	19	56	0.6739	47	55	0.6576	46	55	0.6486	20	23	0.6382	46	60	0.6317
59	28	59	0.6739	47	58	0.6576	49	59	0.6486	21	38	0.6382	4	11	0.6303
60	44	57	0.6739	2	59	0.6563	17	45	0.6473	24	45	0.6382	4	56	0.6303

Appendix 4. Continued.

Acc. no.		Distance	Acc. no.		Distance	Acc. no.		Distance	Acc. no.		Distance	Acc. no.		Distance
12	18	0.6303	2	58	0.6237	4	12	0.6183	33	47	0.6129	19	46	0.6074
18	53	0.6303	14	47	0.6237	4	27	0.6183	46	51	0.6129	23	37	0.6074
19	47	0.6303	16	51	0.6237	5	45	0.6183	55	59	0.6129	26	39	0.6074
22	40	0.6303	19	48	0.6237	7	58	0.6183	8	32	0.6115	29	52	0.6074
22	45	0.6303	22	35	0.6237	13	39	0.6183	8	46	0.6115	32	38	0.6074
23	34	0.6303	22	46	0.6237	14	31	0.6183	11	22	0.6115	33	38	0.6074
31	51	0.6303	23	52	0.6237	14	39	0.6183	12	44	0.6115	33	40	0.6074
37	60	0.6303	29	31	0.6237	16	21	0.6183	16	48	0.6115	51	57	0.6074
39	56	0.6303	32	39	0.6237	16	31	0.6183	20	34	0.6115	52	57	0.6074
43	54	0.6303	32	45	0.6237	18	54	0.6183	23	35	0.6115	2	38	0.6060
47	52	0.6303	41	53	0.6237	19	20	0.6183	25	52	0.6115	3	38	0.6060
48	58	0.6303	49	57	0.6237	22	30	0.6183	25	60	0.6115	4	45	0.6060
3	57	0.6290	1	46	0.6223	24	44	0.6183	27	33	0.6115	4	52	0.6060
4	44	0.6290	4	38	0.6223	24	46	0.6183	27	36	0.6115	11	43	0.6060
4	46	0.6290	6	27	0.6223	27	51	0.6183	29	34	0.6115	12	32	0.6060
22	36	0.6290	6	44	0.6223	43	55	0.6183	32	58	0.6115	13	44	0.6060
24	30	0.6290	11	24	0.6223	4	47	0.6170	33	57	0.6115	14	32	0.6060
26	37	0.6290	13	46	0.6223	6	46	0.6170	43	52	0.6115	14	34	0.6060
26	47	0.6290	14	20	0.6223	6	58	0.6170	43	60	0.6115	15	55	0.6060
28	58	0.6290	15	32	0.6223	10	58	0.6170	4	55	0.6102	16	35	0.6060
35	59	0.6290	18	20	0.6223	12	23	0.6170	9	27	0.6102	19	39	0.6060
2	43	0.6277	19	45	0.6223	14	46	0.6170	11	46	0.6102	20	26	0.6060
4	54	0.6277	22	38	0.6223	16	44	0.6170	12	43	0.6102	21	30	0.6060
11	52	0.6277	22	52	0.6223	19	38	0.6170	13	37	0.6102	25	39	0.6060
13	58	0.6277	26	32	0.6223	22	39	0.6170	13	40	0.6102	28	45	0.6060
20	44	0.6277	31	59	0.6223	24	38	0.6170	15	37	0.6102	36	58	0.6060
20	48	0.6277	39	52	0.6223	27	34	0.6170	16	38	0.6102	38	52	0.6060
22	37	0.6277	2	53	0.6210	37	52	0.6170	16	47	0.6102	41	52	0.6060
22	47	0.6277	6	45	0.6210	44	53	0.6170	25	46	0.6102	41	56	0.6060
24	37	0.6277	10	45	0.6210	1	39	0.6156	29	47	0.6102	2	17	0.6047
26	58	0.6277	11	32	0.6210	2	27	0.6156	29	60	0.6102	2	46	0.6047
27	44	0.6277	11	44	0.6210	3	20	0.6156	33	37	0.6102	6	19	0.6047
27	48	0.6277	14	45	0.6210	7	27	0.6156	41	55	0.6102	6	25	0.6047
39	51	0.6277	16	37	0.6210	11	23	0.6156	2	22	0.6088	7	23	0.6047
39	60	0.6277	16	52	0.6210	17	38	0.6156	2	37	0.6088	10	19	0.6047
47	51	0.6277	17	47	0.6210	20	51	0.6156	2	56	0.6088	12	52	0.6047
6	21	0.6264	18	56	0.6210	21	23	0.6156	4	39	0.6088	14	38	0.6047
8	21	0.6264	18	60	0.6210	22	48	0.6156	10	46	0.6088	15	56	0.6047
11	18	0.6264	20	42	0.6210	23	40	0.6156	15	44	0.6088	19	35	0.6047
12	22	0.6264	25	55	0.6210	23	47	0.6156	17	53	0.6088	21	44	0.6047
12	47	0.6264	26	31	0.6210	27	38	0.6156	20	33	0.6088	23	30	0.6047
13	38	0.6264	32	47	0.6210	50	58	0.6156	24	50	0.6088	23	39	0.6047
15	38	0.6264	4	31	0.6197	8	20	0.6142	27	35	0.6088	23	45	0.6047
20	43	0.6264	4	37	0.6197	14	53	0.6142	30	53	0.6088	25	35	0.6047
21	46	0.6264	15	53	0.6197	19	37	0.6142	32	46	0.6088	29	45	0.6047
22	34	0.6264	16	33	0.6197	23	33	0.6142	41	54	0.6088	30	60	0.6047
1	27	0.6250	18	55	0.6197	24	60	0.6142	45	49	0.6088	31	33	0.6047
4	43	0.6250	19	52	0.6197	25	37	0.6142	1	43	0.6074	57	60	0.6047
9	45	0.6250	21	52	0.6197	25	47	0.6142	2	12	0.6074	2	11	0.6033
11	47	0.6250	23	48	0.6197	27	46	0.6142	4	34	0.6074	2	54	0.6033
12	46	0.6250	24	39	0.6197	7	20	0.6129	6	47	0.6074	3	47	0.6033
18	27	0.6250	24	40	0.6197	9	58	0.6129	8	11	0.6074	4	60	0.6033
20	35	0.6250	24	41	0.6197	17	37	0.6129	8	38	0.6074	5	58	0.6033
23	46	0.6250	25	45	0.6197	18	51	0.6129	11	55	0.6074	9	47	0.6033
30	58	0.6250	25	51	0.6197	25	33	0.6129	14	56	0.6074	19	40	0.6033
31	60	0.6250	29	55	0.6197	26	40	0.6129	15	43	0.6074	22	43	0.6033
40	52	0.6250	32	57	0.6197	27	42	0.6129	15	54	0.6074	25	38	0.6033
56	59	0.6250	34	58	0.6197	27	50	0.6129	16	40	0.6074	26	34	0.6033
2	32	0.6237	35	57	0.6197	31	57	0.6129	17	31	0.6074	31	56	0.6033
12	18	0.6303	2	44	0.6183	33	39	0.6129	17	56	0.6074	35	53	0.6033

Appendix 4. Continued.

Acc. no.		Distance	Acc. no.		Distance	Acc. no.		Distance	Acc. no.		Distance	Acc. no.		Distance
43	56	0.6033	24	49	0.5977	32	40	0.5935	4	14	0.5879	14	44	0.5836
1	11	0.6019	26	53	0.5977	34	39	0.5935	4	48	0.5879	17	44	0.5836
2	34	0.6019	31	52	0.5977	35	60	0.5935	6	11	0.5879	21	22	0.5836
10	39	0.6019	34	53	0.5977	54	57	0.5935	8	25	0.5879	26	35	0.5836
12	19	0.6019	2	18	0.5963	1	35	0.5921	12	25	0.5879	43	47	0.5836
14	43	0.6019	3	46	0.5963	2	39	0.5921	13	41	0.5879	44	55	0.5836
15	41	0.6019	4	17	0.5963	3	22	0.5921	13	56	0.5879	1	40	0.5822
15	47	0.6019	4	18	0.5963	4	25	0.5921	15	60	0.5879	1	41	0.5822
16	41	0.6019	4	30	0.5963	4	40	0.5921	18	24	0.5879	3	32	0.5822
17	40	0.6019	5	38	0.5963	7	22	0.5921	27	49	0.5879	10	22	0.5822
19	36	0.6019	5	47	0.5963	7	25	0.5921	31	46	0.5879	11	16	0.5822
20	25	0.6019	6	22	0.5963	8	53	0.5921	32	42	0.5879	11	53	0.5822
20	50	0.6019	6	43	0.5963	12	29	0.5921	35	38	0.5879	18	22	0.5822
21	55	0.6019	6	52	0.5963	13	20	0.5921	35	54	0.5879	18	52	0.5822
24	36	0.6019	8	19	0.5963	16	30	0.5921	44	60	0.5879	23	43	0.5822
27	30	0.6019	8	41	0.5963	17	30	0.5921	2	40	0.5864	26	52	0.5822
35	52	0.6019	16	43	0.5963	17	32	0.5921	2	60	0.5864	1	30	0.5807
2	19	0.6005	21	35	0.5963	18	23	0.5921	6	23	0.5864	2	28	0.5807
2	52	0.6005	30	56	0.5963	18	26	0.5921	11	25	0.5864	6	18	0.5807
4	22	0.6005	31	53	0.5963	19	41	0.5921	17	48	0.5864	8	30	0.5807
6	53	0.6005	35	56	0.5963	21	43	0.5921	19	42	0.5864	11	19	0.5807
7	19	0.6005	44	56	0.5963	21	54	0.5921	19	43	0.5864	14	48	0.5807
7	24	0.6005	45	48	0.5963	26	48	0.5921	19	50	0.5864	15	35	0.5807
11	54	0.6005	2	31	0.5949	2	14	0.5907	23	44	0.5864	17	34	0.5807
18	29	0.6005	2	45	0.5949	2	55	0.5907	26	44	0.5864	21	41	0.5807
22	50	0.6005	3	21	0.5949	3	58	0.5907	29	46	0.5864	26	55	0.5807
25	40	0.6005	4	28	0.5949	6	31	0.5907	30	55	0.5864	28	47	0.5807
25	41	0.6005	4	51	0.5949	8	22	0.5907	31	42	0.5864	31	38	0.5807
26	46	0.6005	5	46	0.5949	8	39	0.5907	31	54	0.5864	4	35	0.5793
29	35	0.6005	6	12	0.5949	10	11	0.5907	33	46	0.5864	5	22	0.5793
35	55	0.6005	9	38	0.5949	10	41	0.5907	43	51	0.5864	6	39	0.5793
35	58	0.6005	11	50	0.5949	11	29	0.5907	1	38	0.5850	7	29	0.5793
41	60	0.6005	13	30	0.5949	14	54	0.5907	2	13	0.5850	10	21	0.5793
1	22	0.5991	13	53	0.5949	16	39	0.5907	3	27	0.5850	14	37	0.5793
4	13	0.5991	14	35	0.5949	17	39	0.5907	5	19	0.5850	14	51	0.5793
6	37	0.5991	14	55	0.5949	17	54	0.5907	5	37	0.5850	15	52	0.5793
8	31	0.5991	16	36	0.5949	23	38	0.5907	5	39	0.5850	16	50	0.5793
8	47	0.5991	20	36	0.5949	25	48	0.5907	10	43	0.5850	17	43	0.5793
13	43	0.5991	21	42	0.5949	26	38	0.5907	11	48	0.5850	17	51	0.5793
14	41	0.5991	23	36	0.5949	26	56	0.5907	12	54	0.5850	21	48	0.5793
15	31	0.5991	24	43	0.5949	30	52	0.5907	13	31	0.5850	29	51	0.5793
15	39	0.5991	28	31	0.5949	2	24	0.5893	14	60	0.5850	30	51	0.5793
17	60	0.5991	1	19	0.5935	2	25	0.5893	17	52	0.5850	31	36	0.5793
19	30	0.5991	1	21	0.5935	2	47	0.5893	18	25	0.5850	32	51	0.5793
19	44	0.5991	1	31	0.5935	4	19	0.5893	20	28	0.5850	34	45	0.5793
27	39	0.5991	1	47	0.5935	5	27	0.5893	21	53	0.5850	36	40	0.5793
27	41	0.5991	2	23	0.5935	6	30	0.5893	25	44	0.5850	41	46	0.5793
29	37	0.5991	6	54	0.5935	6	55	0.5893	26	54	0.5850	44	52	0.5793
30	54	0.5991	8	43	0.5935	9	19	0.5893	28	53	0.5850	44	54	0.5793
31	58	0.5991	8	45	0.5935	12	16	0.5893	31	55	0.5850	48	54	0.5793
32	41	0.5991	9	46	0.5935	12	30	0.5893	32	37	0.5850	1	18	0.5778
36	47	0.5991	10	38	0.5935	12	58	0.5893	32	60	0.5850	1	20	0.5778
52	58	0.5991	10	47	0.5935	15	46	0.5893	49	58	0.5850	1	32	0.5778
3	37	0.5977	11	30	0.5935	16	46	0.5893	54	58	0.5850	3	25	0.5778
8	12	0.5977	14	40	0.5935	17	46	0.5893	3	19	0.5836	3	40	0.5778
14	30	0.5977	15	40	0.5935	18	33	0.5893	3	43	0.5836	4	24	0.5778
15	34	0.5977	21	29	0.5935	21	50	0.5893	4	41	0.5836	6	14	0.5778
17	55	0.5977	21	32	0.5935	29	48	0.5893	9	22	0.5836	7	45	0.5778
22	42	0.5977	23	41	0.5935	31	43	0.5893	9	39	0.5836	8	34	0.5778
22	49	0.5977	25	30	0.5935	36	46	0.5893	12	27	0.5836	8	44	0.5778

Appendix 4. Continued.

Acc. no.		Distance	Acc. no.		Distance	Acc. no.		Distance	Acc. no.		Distance	Acc. no.		Distance
9	40	0.5778	11	41	0.5720	34	54	0.5676	5	25	0.5617	2	29	0.5557
12	55	0.5778	12	35	0.5720	35	51	0.5676	5	31	0.5617	4	15	0.5557
13	32	0.5778	12	53	0.5720	46	49	0.5676	7	12	0.5617	12	42	0.5557
14	29	0.5778	13	55	0.5720	1	53	0.5662	8	52	0.5617	16	42	0.5557
19	21	0.5778	16	18	0.5720	3	24	0.5662	11	36	0.5617	28	44	0.5557
21	60	0.5778	17	20	0.5720	7	11	0.5662	14	42	0.5617	29	49	0.5557
28	56	0.5778	17	35	0.5720	7	47	0.5662	15	21	0.5617	36	43	0.5557
29	33	0.5778	21	25	0.5720	8	55	0.5662	28	32	0.5617	37	48	0.5557
31	45	0.5778	21	56	0.5720	9	37	0.5662	34	42	0.5617	56	57	0.5557
3	14	0.5764	26	30	0.5720	10	32	0.5662	34	56	0.5617	3	11	0.5542
4	33	0.5764	28	39	0.5720	10	35	0.5662	37	46	0.5617	6	29	0.5542
5	40	0.5764	31	39	0.5720	11	49	0.5662	38	48	0.5617	8	17	0.5542
6	40	0.5764	34	46	0.5720	12	34	0.5662	1	16	0.5602	8	29	0.5542
6	56	0.5764	41	51	0.5720	13	29	0.5662	1	17	0.5602	10	29	0.5542
6	60	0.5764	50	54	0.5720	13	48	0.5662	2	50	0.5602	14	27	0.5542
10	25	0.5764	1	24	0.5706	28	37	0.5662	4	50	0.5602	25	42	0.5542
10	31	0.5764	4	16	0.5706	28	43	0.5662	6	17	0.5602	28	51	0.5542
10	40	0.5764	6	28	0.5706	29	44	0.5662	7	21	0.5602	30	33	0.5542
11	34	0.5764	7	53	0.5706	46	48	0.5662	7	52	0.5602	30	46	0.5542
11	58	0.5764	8	56	0.5706	2	26	0.5647	7	56	0.5602	31	41	0.5542
17	41	0.5764	9	23	0.5706	2	36	0.5647	8	23	0.5602	33	58	0.5542
21	36	0.5764	13	24	0.5706	7	26	0.5647	8	26	0.5602	35	44	0.5542
25	36	0.5764	13	54	0.5706	7	55	0.5647	9	11	0.5602	37	41	0.5542
26	41	0.5764	14	52	0.5706	9	20	0.5647	9	41	0.5602	37	43	0.5542
28	46	0.5764	23	50	0.5706	11	27	0.5647	10	23	0.5602	37	49	0.5542
29	39	0.5764	26	60	0.5706	20	52	0.5647	11	56	0.5602	42	43	0.5542
33	43	0.5764	27	43	0.5706	23	49	0.5647	15	48	0.5602	45	50	0.5542
42	53	0.5764	28	55	0.5706	28	34	0.5647	20	32	0.5602	48	55	0.5542
44	51	0.5764	31	44	0.5706	28	35	0.5647	25	50	0.5602	50	56	0.5542
4	7	0.5749	32	43	0.5706	28	48	0.5647	26	36	0.5602	53	58	0.5542
6	34	0.5749	35	45	0.5706	29	38	0.5647	29	30	0.5602	3	17	0.5527
11	42	0.5749	40	46	0.5706	29	40	0.5647	29	36	0.5602	3	18	0.5527
13	23	0.5749	42	46	0.5706	29	41	0.5647	35	46	0.5602	4	29	0.5527
13	27	0.5749	2	41	0.5691	36	37	0.5647	39	45	0.5602	5	21	0.5527
13	35	0.5749	3	31	0.5691	36	39	0.5647	40	49	0.5602	7	46	0.5527
15	36	0.5749	3	39	0.5691	58	60	0.5647	42	56	0.5602	8	28	0.5527
18	32	0.5749	4	49	0.5691	1	12	0.5632	48	53	0.5602	8	54	0.5527
24	42	0.5749	6	48	0.5691	1	34	0.5632	2	16	0.5587	9	29	0.5527
25	43	0.5749	9	24	0.5691	1	44	0.5632	7	16	0.5587	10	24	0.5527
28	60	0.5749	9	31	0.5691	5	20	0.5632	11	26	0.5587	11	17	0.5527
30	32	0.5749	10	30	0.5691	6	35	0.5632	11	35	0.5587	13	19	0.5527
1	37	0.5735	15	33	0.5691	7	39	0.5632	16	49	0.5587	13	25	0.5527
2	30	0.5735	17	33	0.5691	9	43	0.5632	18	19	0.5587	20	49	0.5527
2	35	0.5735	19	49	0.5691	10	12	0.5632	21	27	0.5587	21	26	0.5527
6	24	0.5735	20	53	0.5691	10	20	0.5632	31	48	0.5587	21	34	0.5527
6	41	0.5735	28	54	0.5691	12	48	0.5632	34	40	0.5587	34	37	0.5527
12	41	0.5735	34	55	0.5691	14	33	0.5632	35	42	0.5587	44	47	0.5527
13	52	0.5735	38	41	0.5691	15	30	0.5632	35	43	0.5587	1	29	0.5512
13	60	0.5735	41	45	0.5691	21	49	0.5632	4	42	0.5572	5	16	0.5512
15	51	0.5735	2	33	0.5676	28	38	0.5632	6	26	0.5572	5	18	0.5512
20	56	0.5735	3	44	0.5676	31	47	0.5632	7	38	0.5572	5	23	0.5512
26	50	0.5735	4	36	0.5676	32	48	0.5632	7	60	0.5572	7	54	0.5512
34	47	0.5735	8	40	0.5676	33	44	0.5632	10	44	0.5572	28	41	0.5512
34	52	0.5735	10	37	0.5676	42	54	0.5632	12	26	0.5572	32	33	0.5512
34	60	0.5735	12	50	0.5676	55	57	0.5632	13	34	0.5572	32	44	0.5512
36	38	0.5735	18	28	0.5676	1	25	0.5617	18	36	0.5572	35	36	0.5512
50	53	0.5735	20	54	0.5676	2	48	0.5617	28	52	0.5572	42	47	0.5512
2	51	0.5720	20	60	0.5676	3	23	0.5617	38	49	0.5572	55	58	0.5512
8	35	0.5720	29	43	0.5676	4	23	0.5617	42	45	0.5572	3	53	0.5497
9	25	0.5720	29	50	0.5676	5	24	0.5617	2	15	0.5557	7	14	0.5497

Appendix 4. Continued.

Acc. no.		Distance	Acc. no.		Distance	Acc. no.		Distance	Acc. no.		Distance	Acc. no.		Distance
8	60	0.5497	8	51	0.5436	3	41	0.5359	14	22	0.5280	20	55	0.5200
9	30	0.5497	13	51	0.5436	5	14	0.5359	16	28	0.5280	39	43	0.5200
11	14	0.5497	14	21	0.5436	5	30	0.5359	18	49	0.5280	1	28	0.5184
17	24	0.5497	14	24	0.5436	5	32	0.5359	19	26	0.5280	1	52	0.5184
17	50	0.5497	15	42	0.5436	5	44	0.5359	32	52	0.5280	3	26	0.5184
18	38	0.5497	18	43	0.5436	7	51	0.5359	34	38	0.5280	7	15	0.5184
26	42	0.5497	26	43	0.5436	10	16	0.5359	34	49	0.5280	9	14	0.5184
28	30	0.5497	31	34	0.5436	11	28	0.5359	36	52	0.5280	13	21	0.5184
33	41	0.5497	34	41	0.5436	13	26	0.5359	36	54	0.5280	14	25	0.5184
35	37	0.5497	39	46	0.5436	14	26	0.5359	3	60	0.5264	15	24	0.5184
39	48	0.5497	1	13	0.5421	15	27	0.5359	5	17	0.5264	23	28	0.5184
41	48	0.5497	1	48	0.5421	32	55	0.5359	8	13	0.5264	28	36	0.5184
42	55	0.5497	6	13	0.5421	41	49	0.5359	10	17	0.5264	33	50	0.5184
45	46	0.5497	8	14	0.5421	53	57	0.5359	30	47	0.5264	35	40	0.5184
2	42	0.5482	14	18	0.5421	3	13	0.5343	30	48	0.5264	36	56	0.5184
3	52	0.5482	14	36	0.5421	13	22	0.5343	42	60	0.5264	44	49	0.5184
9	21	0.5482	17	29	0.5421	14	50	0.5343	3	15	0.5248	3	28	0.5168
12	17	0.5482	18	46	0.5421	18	45	0.5343	7	44	0.5248	3	50	0.5168
13	18	0.5482	38	42	0.5421	30	38	0.5343	11	13	0.5248	12	14	0.5168
15	18	0.5482	40	43	0.5421	30	45	0.5343	14	49	0.5248	33	48	0.5168
24	28	0.5482	41	43	0.5421	32	50	0.5343	15	49	0.5248	36	55	0.5168
25	49	0.5482	41	50	0.5421	41	44	0.5343	22	29	0.5248	50	52	0.5168
31	37	0.5482	47	49	0.5421	48	52	0.5343	30	43	0.5248	9	17	0.5152
31	49	0.5482	3	30	0.5405	6	16	0.5327	35	50	0.5248	10	56	0.5152
32	35	0.5482	5	41	0.5405	8	48	0.5327	1	26	0.5232	11	15	0.5152
32	36	0.5482	8	37	0.5405	10	26	0.5327	5	12	0.5232	12	28	0.5152
36	53	0.5482	12	56	0.5405	14	23	0.5327	5	35	0.5232	15	50	0.5152
39	49	0.5482	18	42	0.5405	18	34	0.5327	7	28	0.5232	17	21	0.5152
41	47	0.5482	23	42	0.5405	18	48	0.5327	7	41	0.5232	30	50	0.5152
42	52	0.5482	26	51	0.5405	30	36	0.5327	10	14	0.5232	34	51	0.5152
43	49	0.5482	32	56	0.5405	31	40	0.5327	10	53	0.5232	35	49	0.5152
3	12	0.5467	46	50	0.5405	33	35	0.5327	36	48	0.5232	39	44	0.5152
4	26	0.5467	47	48	0.5405	34	48	0.5327	37	42	0.5232	56	58	0.5152
5	11	0.5467	48	56	0.5405	38	39	0.5327	38	43	0.5232	1	55	0.5136
5	43	0.5467	2	7	0.5390	38	47	0.5327	39	47	0.5232	12	15	0.5136
6	51	0.5467	3	54	0.5390	39	50	0.5327	41	42	0.5232	15	26	0.5136
9	16	0.5467	10	18	0.5390	43	45	0.5327	42	51	0.5232	18	44	0.5136
13	42	0.5467	10	34	0.5390	45	47	0.5327	3	29	0.5217	22	28	0.5136
13	50	0.5467	13	33	0.5390	50	60	0.5327	3	55	0.5217	28	49	0.5136
22	26	0.5467	13	36	0.5390	9	32	0.5312	6	49	0.5217	28	50	0.5136
32	49	0.5467	17	36	0.5390	12	49	0.5312	9	18	0.5217	30	41	0.5136
32	54	0.5467	43	50	0.5390	18	47	0.5312	12	60	0.5217	37	39	0.5136
43	46	0.5467	49	54	0.5390	40	48	0.5312	16	25	0.5217	39	41	0.5136
43	48	0.5467	49	56	0.5390	57	59	0.5312	28	33	0.5217	51	52	0.5136
50	55	0.5467	1	56	0.5374	3	56	0.5296	30	42	0.5217	1	60	0.5120
51	58	0.5467	3	16	0.5374	5	15	0.5296	30	49	0.5217	5	52	0.5120
58	59	0.5467	3	34	0.5374	5	29	0.5296	34	43	0.5217	7	32	0.5120
1	23	0.5451	3	48	0.5374	6	36	0.5296	35	47	0.5217	9	53	0.5120
2	49	0.5451	7	17	0.5374	7	43	0.5296	42	48	0.5217	10	28	0.5120
6	33	0.5451	9	35	0.5374	8	18	0.5296	48	60	0.5217	10	52	0.5120
11	60	0.5451	9	44	0.5374	17	23	0.5296	49	53	0.5217	14	19	0.5120
12	36	0.5451	17	42	0.5374	26	33	0.5296	5	53	0.5200	15	25	0.5120
17	18	0.5451	18	39	0.5374	30	34	0.5296	6	15	0.5200	17	49	0.5120
28	40	0.5451	18	41	0.5374	31	50	0.5296	7	37	0.5200	22	27	0.5120
35	48	0.5451	26	49	0.5374	32	53	0.5296	8	15	0.5200	36	50	0.5120
39	42	0.5451	33	52	0.5374	44	46	0.5296	8	33	0.5200	42	44	0.5120
1	54	0.5436	35	39	0.5374	50	51	0.5296	10	48	0.5200	5	48	0.5103
3	35	0.5436	36	41	0.5374	6	42	0.5280	13	16	0.5200	7	42	0.5103
6	50	0.5436	1	14	0.5359	9	12	0.5280	16	27	0.5200	8	36	0.5103
8	24	0.5436	1	15	0.5359	9	26	0.5280	17	26	0.5200	30	31	0.5103

Appendix 4. Continued.

Acc. no.		Distance	Acc. no.		Distance	Acc. no.		Distance	Acc. no.		Distance	Acc. no.		Distance
30	37	0.5103	49	55	0.4987	3	49	0.4834	10	42	0.4641	13	14	0.4246
40	41	0.5103	1	6	0.4971	9	50	0.4834	15	28	0.4641	49	50	0.4246
4	10	0.5087	3	42	0.4971	9	56	0.4834	25	26	0.4641	3	6	0.4226
9	48	0.5087	5	34	0.4971	10	13	0.4834	38	44	0.4641	12	51	0.4226
18	50	0.5087	12	13	0.4971	11	21	0.4834	48	49	0.4641	53	54	0.4226
24	27	0.5087	14	16	0.4971	38	45	0.4834	13	17	0.4623	7	9	0.4207
26	28	0.5087	15	23	0.4971	42	50	0.4834	23	26	0.4623	51	56	0.4207
34	50	0.5087	15	29	0.4971	47	50	0.4834	5	49	0.4605	9	36	0.4187
35	41	0.5087	21	28	0.4971	49	51	0.4834	7	34	0.4605	33	60	0.4187
36	60	0.5087	22	25	0.4971	52	60	0.4834	9	13	0.4605	5	33	0.4167
40	44	0.5087	23	27	0.4971	3	36	0.4817	12	21	0.4605	48	50	0.4147
5	26	0.5070	30	44	0.4971	7	13	0.4817	24	29	0.4605	52	55	0.4106
10	55	0.5070	34	35	0.4971	13	28	0.4817	43	44	0.4605	15	16	0.4086
17	27	0.5070	34	44	0.4971	15	19	0.4817	9	42	0.4586	9	33	0.4024
21	51	0.5070	39	40	0.4971	18	40	0.4817	26	29	0.4586	53	56	0.4024
27	29	0.5070	40	47	0.4971	9	60	0.4800	52	54	0.4586	8	9	0.3982
38	50	0.5070	2	8	0.4954	37	50	0.4800	10	49	0.4568	12	33	0.3961
54	60	0.5070	4	8	0.4954	1	4	0.4782	17	28	0.4568	1	9	0.3898
1	7	0.5054	21	33	0.4954	1	42	0.4782	6	10	0.4550	1	5	0.3876
1	51	0.5054	25	29	0.4954	2	6	0.4782	10	36	0.4550	3	9	0.3876
2	10	0.5054	31	32	0.4954	5	54	0.4782	11	51	0.4550	55	56	0.3811
4	5	0.5054	33	42	0.4954	10	50	0.4782	16	23	0.4550	51	60	0.3789
6	7	0.5054	3	7	0.4937	17	22	0.4782	16	29	0.4550	5	10	0.3700
8	49	0.5054	5	13	0.4937	17	25	0.4782	18	30	0.4531	8	10	0.3700
13	49	0.5054	7	31	0.4937	18	37	0.4782	3	4	0.4513	3	5	0.3585
27	28	0.5054	7	40	0.4937	33	49	0.4782	13	15	0.4513	1	10	0.3562
29	42	0.5054	18	35	0.4937	36	42	0.4782	52	56	0.4513	5	9	0.3191
33	54	0.5054	32	34	0.4937	36	51	0.4782	3	8	0.4494	11	12	0.3191
8	16	0.5037	33	34	0.4937	46	47	0.4782	7	10	0.4494	9	10	0.3085
10	15	0.5037	33	53	0.4937	51	53	0.4782	30	40	0.4494	33	51	0.2975
22	24	0.5037	40	42	0.4937	51	55	0.4782	1	8	0.4476			
28	42	0.5037	51	54	0.4937	19	22	0.4765	6	9	0.4457			
30	35	0.5037	2	5	0.4920	1	49	0.4747	53	60	0.4457			
34	36	0.5037	5	28	0.4920	1	50	0.4747	2	3	0.4438			
36	44	0.5037	7	33	0.4920	3	33	0.4747	6	8	0.4438			
40	50	0.5037	9	34	0.4920	5	42	0.4747	9	51	0.4438			
44	48	0.5037	9	55	0.4920	7	35	0.4747	10	33	0.4438			
7	8	0.5021	10	54	0.4920	8	42	0.4747	26	27	0.4438			
19	29	0.5021	10	60	0.4920	10	51	0.4747	33	56	0.4420			
30	39	0.5021	14	28	0.4920	23	29	0.4730	52	53	0.4420			
48	51	0.5021	16	22	0.4920	37	45	0.4730	37	40	0.4401			
2	9	0.5004	3	51	0.4903	49	52	0.4730	5	51	0.4382			
4	9	0.5004	5	55	0.4903	5	60	0.4712	7	18	0.4382			
9	28	0.5004	9	54	0.4903	16	24	0.4712	11	33	0.4382			
9	52	0.5004	16	17	0.4903	24	26	0.4712	22	23	0.4382			
19	24	0.5004	37	47	0.4903	25	27	0.4712	54	55	0.4382			
19	28	0.5004	42	49	0.4903	5	6	0.4694	55	60	0.4382			
24	25	0.5004	5	50	0.4886	37	38	0.4694	9	49	0.4363			
33	55	0.5004	5	56	0.4886	5	7	0.4677	33	36	0.4363			
38	40	0.5004	7	48	0.4886	7	30	0.4677	54	56	0.4363			
7	36	0.4987	28	29	0.4886	23	25	0.4677	56	60	0.4363			
8	50	0.4987	37	44	0.4886	36	49	0.4677	1	3	0.4343			
9	15	0.4987	7	49	0.4869	1	33	0.4659	3	10	0.4343			
15	22	0.4987	14	17	0.4869	1	36	0.4659	57	58	0.4324			
16	19	0.4987	16	26	0.4869	7	50	0.4659	17	19	0.4305			
18	31	0.4987	25	28	0.4869	14	15	0.4659	2	4	0.4285			
19	23	0.4987	40	45	0.4869	15	17	0.4659	5	8	0.4285			
19	27	0.4987	49	60	0.4869	19	25	0.4659	53	55	0.4285			
44	45	0.4987	31	35	0.4852	1	2	0.4641	23	24	0.4266			
44	50	0.4987	38	46	0.4852	4	6	0.4641	5	36	0.4246			

Note: N = 1770; Mean = 0.5731; Std. deviation = 6.15; SE = 1.46; T-value = 392.37***; CV (%) = 0.11