ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

MORPHOLOGICAL, AGRONOMIC AND BIOCHEMICAL CHARACTERIZATION OF *TRIFOLIUM STEUDNERI* SCHWEINF., AN INDIGENOUS SPECIES IN ETHIOPIA

A thesis submitted to the Department of Biology in partial fulfillment of the requirements for the Degree of Masters of Science in Biology (Applied Genetics).

By: Yetneberk Ayalew

Advisors: **Dr. Jean Hanson (ILRI)**
Dr. Ensermu Kelbessa (AAU)

October 2002

ACKNOWLEDGEMENTS

First of all I wish to express my sincere gratitude to Dr. Jean Hanson, who gave me the opportunity to start and finish this project at the International Livestock Research Institute (ILRI). Her insistent demand for precision and quality will always remain a standard to work by. Her comments and suggestions were so constructive and supportive. Many thanks Jean.

I would also like to thank Dr. Ensermu Kelbessa for sharing his rich experience during the fieldwork of morphological studies. His cooperative actions in facilitating my needs from the Addis Ababa University (AAU) are unforgettable.

Acknowledgement is also to Ato Getahun from the Institute of Biodiversity Conservation and Research (IBCR), forage division. Thank you for encouraging me to hold on when things seemed so difficult for me.

During my fieldwork at ILRI-Debre Zeit, it is my pleasure to forward my thanks for those who encouraged and helped me in one way or another. Especially, I would like to thank Ato Abate, Ato Asebe, Ato Yersaw, Ato Fetene and all field assistants from the seed unit. Without their assistance and reliability, the fieldwork would not have been possible. Lot's of thanks to W/t Menberework, from the computer unit at ILRI-Debre Zeit, in guiding me how to organize my data and write up.

My thanks also go to all the staff in the forage genetic resource project, ILRI head quarter for their help and encouragement during the lab work. Especially, I would like to thank Ato Girma, in guiding me through the isozyme analysis.

For their technical and statistical analysis support, I would like to thank Ato Yemane from IBCR, Ato Medhane from AAU and Ato Zerihun from ILRI.

I would like to extend my special thanks to all my friends and relatives who encouraged and supported me to complete my graduate studies.

Finally I would like to acknowledge my mother, father and sisters for their priceless support in many aspects. It would really have been impossible without them.

Above all thanks to God almighty.

TABLE OF CONTENTS

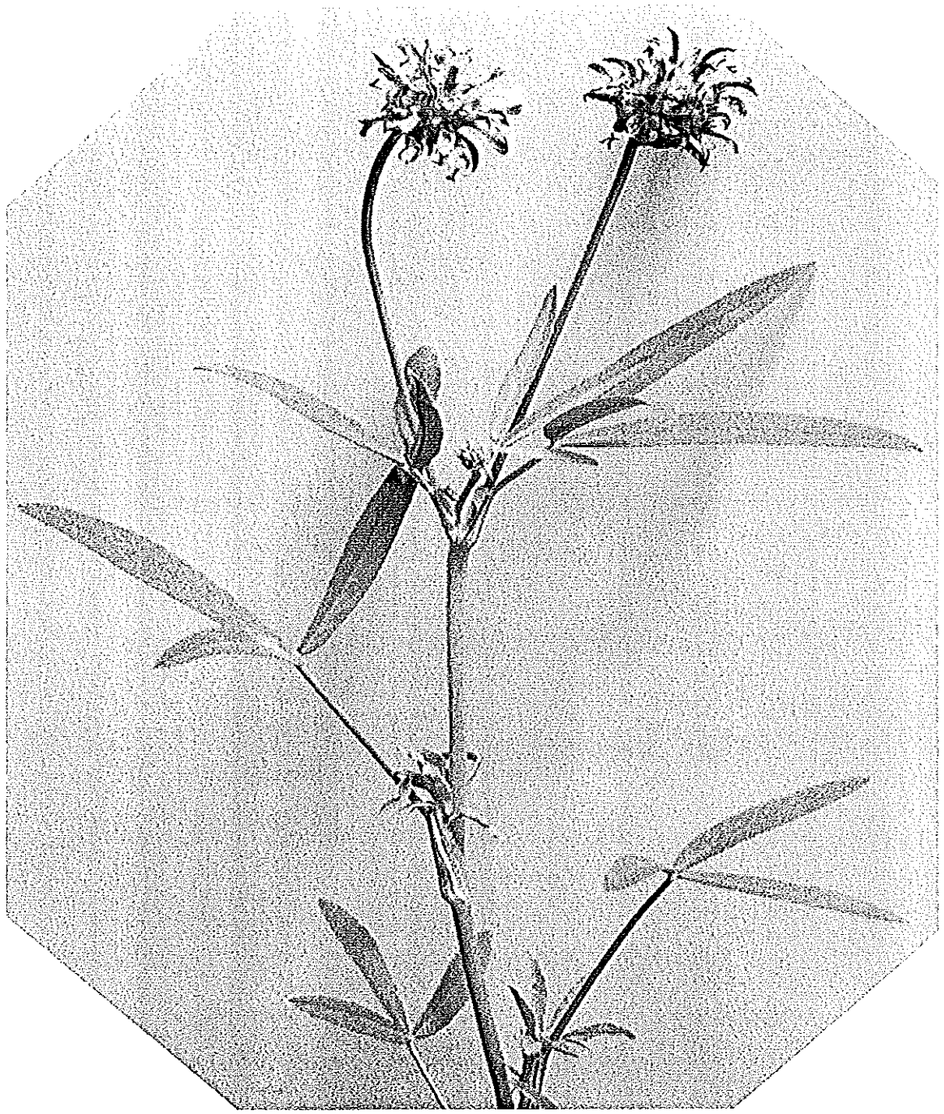
LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF GIS-MAPS

Trifolium steudneri Schweinf.



ABSTRACT

Characterization of forage germplasm is an essential prerequisite for use of forage genetic resources. Fifty accessions of *Trifolium steudneri* Schweinf. were studied for 14 morphological quantitative characters, 9 morphological qualitative characters and 6 agronomic characters. Spatial distribution of collection sites of accessions was checked using DIVA-GIS. Cluster analysis grouped the accessions into six groups, where the sixth group had only one member, accession number 9452. A scatter plot made from the first and second factors of the factor analysis, which explains 33% of the total variation, and also the isozyme analysis indicated this accession to be an outlier. A high correlation was observed between characters such as days to 50% flowering and 75% maturity, and leaf width and stipule length. Accession number 9452 was found to show a higher value of Mahalanobis distance, showing this accession is distantly related to the others. Taking eight agronomic characters, accessions were grouped into three groups of high, medium and low productivity. The percentage frequency of phenotypic classes of the accessions for the regions and altitude group and respective chi-square values for eight qualitative characters were calculated. The overall diversity index was calculated to be 0.40 ± 0.07. Higher heritability values were observed for most of the quantitative morphological characters except for stem thickness and flower width. Four enzyme systems, Peroxidase, Acid phosphatase, α-Esterase and β-Esterase, were used in the isozyme study, indicating the percentage of polymorphic loci ranges from 64.7 to 47.1. The isozyme clustering was found to match with the morphological clustering.

Key words: Characterization, *Trifolium steudneri*, cluster analysis, factor analysis, isozyme analysis, heritability, polymorphic loci, GIS

1. INTRODUCTION

Characterization of forage germplasm is an essential prerequisite for use of forage genetic resources. Scientists should know what variation exists in gene bank collections. The grouping of accessions resulting from characterization will facilitate further evaluation and use. Agronomists can select accessions from different groups to test a wide variety of genetic material, or can limit themselves to evaluating accessions from one group with characteristics in which they are interested (Wouw *et al.*, 1999).

Trifolium is a genus closely related to the genera *Trigonella, Medicago* and *Melilotus*. Its range of distribution extends throughout the temperate and subtropical regions of the globe. Forage legumes with wide distribution are found in the genera *Medicago* and *Trifolium*.

The Mediterranean, the Eritreo-Arabian region (plus its periphery) and the Americas are the three major areas of origin of the genus *Trifolium* (Zohary, 1972). About 150 species of the genus are found in the eastern Mediterranean region, which is the largest center of diversity. The center of distribution of *Trifolium* in southern Sahara comprises central Ethiopia, Somalia, Eritrea, Kenya, Saudi Arabia, Tanzania and Uganda. This is the largest center of diversity of *Trifolium*, in the south of the Sahara (Akundabweni, 1984a).

As a legume, *Trifolium* species present in arable land can revitalize the soil by increasing the nitrogen content and support livestock on poor soil (Norris and Mannetje, 1964). It is also indicated that, inter-cropping clovers in wheat has potential in the African highlands (Kahurananga, 1991). Legumes in general are now regarded as cheap sources of nitrogen,

as sources of fossil fuels required to produce commercial fertilizer are shrinking (Isermann, 1983).

Even though, the introduction of forage legumes is constrained by periodic frosts, extended dry seasons and extensive areas of seasonally water-logged black clay soil (Vertisols) low in pH and available P (Kahurananga, 1991), in Africa many pasture innovations still involve introductions of exotic forage crops, although, as mentioned by Akundabweni (1984b), the "back wood" of the continent might well offer suitable alternatives. Also Akundabweni (1984a; cited Harlan, 1980), states that the use of native forage resources present problems unlike those encountered with improved species: the lack of knowledge about their biology, cultural demands, palatability and nutritional value has so far limited their use. Diversity studies of those native forage genetic resources have not been carried out which also hampered their practical use in forage development strategies for the betterment of the livestock sector in Africa.

In terms of forage production and nutritive value, the clovers rank second to *Medicago sativa* in the United States, Europe, Australia and New Zealand. However, only 4% of the species are agriculturally important (Evans, 1967). The small number of cultivated species in this group is due to its recent domestication (Akundabweni, 1984a).

Legumes more than grasses, appear overlooked by plant explorers in east Africa, although it seems that the mesophytic environment of the region possesses many important genera (Humphreys, 1981). As a result, little is known about the potential forage value of Ethiopia's native clovers. What is certain is that Ethiopia has by far the largest

representation of the genus *Trifolium* in Africa. Over 30 of the clover species found in the eastern, central and West Africa highlands and in parts of southern Africa are also present in Ethiopia. Eight other species are thought to be endemic to Ethiopia (Thulin, 1976), making a total of around 32 *Trifolium* species present in the country.

The focus of this research will be on characterizing *Trifolium steudneri*, which is one of the most widely distributed indigenous *Trifolium* species in Ethiopia. It is an annual species as described in the flora of Ethiopia (Thulin and Hunde, 1989) and as mentioned by Zohary (1972) whose propagation is wholly dependent on its highly specialized seed production.

1.2 OBJECTIVES OF THE RESEARCH

The specific objectives of the research are:

- To test the hypothesis that there is no variation between accessions.
- To conduct a characterization study on *T. steudneri* and assess its diversity.
- To cluster similar populations of *T. steudneri* and identify more productive genotypes for breeding purposes.
- To study the relationship between morphological and biochemical characters.
- To assess potential as a forage crop by studying some key agronomic characters.

2. LITERATURE REVIEW

2.1 ORIGIN AND DISTRIBUTION OF THE GENUS *TRIFOLIUM*

2.1.1 Family Fabaceae

During the great expansion of the angiosperms, Fabaceae (Leguminoseae) was one of the early plant families, which is thought to originate in the cretaceous times. Eighty two percent of the genera in this family are tropical in origin (Whyte, 1974). The family comprises about 650 genera and 18000 species, widely distributed over the world (Thulin and Hunde, 1989). *Medicago* and *Trifolium* are those genera, which contain forage legumes with wide distribution (White *et al.*, 1973).

The family Fabaceae is further divided into three sub-families: Caesalpinioideae, Mimosoideae and Papilionoideae. The three subfamilies are separated on the basis of the feature of leaves, flowers and seeds. According to Thulin and Hunde (1989), leaves of Mimosoideae are almost bipinnate. They are also known to have regular flowers. The petals are valvate in bud and often united at the base. The stamens are as many as the petals or twice as many or numerous. The seeds are normally areole (an area on each face of a seed bounded by a fine line or crack in the testa) on each side. This subfamily has about 50 genera and 2800 species, which are mostly tropical and subtropical, especially numerous in the southern hemisphere.

Subfamily Caesalpinioideae usually has an adaxial (upper) petal overlapped by the adjacent lateral petals when these are present. The sepals and stamens are often free. The seeds with radicle are usually straight. This subfamily has about 150 genera and 2500 species, mostly tropical and subtropical.

Subfamily Papilionoideae has an adaxial petal (standard) outside the adjacent lateral petals (wings). Sepals are united at the base, while the stamens are usually variously united. Seeds with radicle are usually curved. This subfamily has about 440 genera and 12000 species, widely distributed in most parts of the world, but with the greatest diversity in tropical and subtropical regions. The genus *Trifolium* is found in this subfamily.

Only *Leucaena* and *Desmanthus* species in the Mimosoideae have known forage value. Of the major taxa, the Papilionoideae contains the highest number of tropical species and many of the most promising tropical plant introductions have come from this group. In this subfamily, genera like *Cajanus, Calopogonium, Clitoria, Centrosema, Neonotonia, Lablab, Macroptilium, Macrotyloma* and *Pueraria* have important forage cultivars. In the subfamily Papilionoideae, 91 genera are reported to be found in tropical Africa with about 1000 species represented in east Africa alone. In this taxon, the tribe Genisteae contains *Lotonis, Crotalaria, Lupinus* and *Argyrolobium*. The Trifoliaeae contains *Trigonella, Medicago, Melilotus and Trifolium*. It is reported that most of these genera have species, which are largely unimproved for use in their areas of distribution (Akundabweni, 1984b).

There is a limited distribution for certain members of the Caesalpinioideae in Africa, now thought to have become temperate legumes (Humphreys, 1981), but with origin from the true relic tropical types. On the other hand, in some members of Trifolieae movement is thought to have taken place in the opposite direction, from the temperate to the tropics. Such migration to the equator is believed to occur through the path provided by the tropical highlands (Humphreys, 1981; Akundabweni, 1984a). Some members in the genus

Trifolium, which are found in large number in the East African highlands, can be an example for such migration.

2.1.2 Genus *Trifolium*

The origin of the genus is traced back to the ancestral section Lotodeae, which is assumed to have existed from Neogene times (Zohary, 1972). The Mediterranean region is generally believed to be the centre of the genus *Trifolium* (Eeghen, 1984). But it is also believed that the Eritreo-Arabian region and the Americas can also be the major areas of origin.

According to Akundabweni (1984a), there are eight sections of the genus *Trifolium* out of which the African clovers belong to four of the sections. The eastern Mediterranean region is believed to be the largest centre of diversity, having 150 species representing seven of the eight sections of the genus. The largest centre of diversity in the southern Sahara includes central Ethiopia (from where most of the accession for this study came from), Somalia, Eritrea, Saudi Arabia, Kenya, Tanzania and Uganda.

This is because of the fact that some members of the genus migrated into the East African highlands by the Afro-alpine Mediterranean line, while the Mediterranean region provides the major centre of differentiation of clover (Akundabweni, 1984a).

Eeghen (1984; cited Harlan, 1969) proposed that Ethiopia is a secondary centre of diversity of *Trifolium* after the Mediterranean area. Whatever the case may be, the highlands of East Africa are very rich in *Trifolium* species. *Trifolium* is the predominant genus in this region, with a total of 40 species. Out of these 32 are found in the Ethiopian highlands where 8 of

them are endemic, being only confined in the highlands of Ethiopia (Thulin and Hunde, 1989).

From the total area of Ethiopia, which is about 1.22 million km^2, the highland occupies 46.7%, or 489,520 km^2, which approximates the area of all other African highlands combined. According to Jahnke (1982), the tropical highlands are defined as areas, which are at least 1500m above sea level, and according to Sanchez (1976) they are areas with a mean daily temperature of less than 20^0C during the growing season. Eeghen (1984) mentioned these areas to show big daily temperature fluctuations, and at the higher altitudes night frost is a common phenomena. Annual rainfall in the highlands of Ethiopia is between 500 and 2000mm, which mostly fall in two rainy seasons, the short rainy season (Belg) and the long rainy season (Meher).

Areas of the Ethiopian highlands, characterized mostly by gentle topography and possessing Vertisols, are mainly used as marginal grazing lands and are rarely cultivated because of problems of poor drainage. Vertisols are deep shrink-swell clay soils with marked waterlogging during the growing season. Vertisols are sometimes referred to as 'black cotton soils' due to their dark color which in fact is not related to the amount of organic matter content but may be due to the presence of diffused humus under alkaline to neutral conditions. Reduced iron compounds may also cause the dark color and are less likely to bind phosphorus (Young, 1980). Calcium and magnesium are normally abundant in these soils whereas potassium and available phosphorus may be low. The low soil permeability and high cations exchange capacity shows that fertilizer nutrients are well

retained and with proper management many Vertisols are capable of supporting annual cropping at moderate to high yields (Young, 1980).

The characteristic expansion and contraction of Vertisols when wet or dry causes large cracks in the ground, which develop during the dry season. Vertisols do not usually support tree vegetation since the cracking of the soil can be difficult for the rooting behavior of trees. Some indigenous plants survive better on these soils by rapidly completing maturation before the soil cracking occurs so that they escape the potential root damage. This may be particularly true for annual species (Akundabweni, 1984a), including annual *Trifolium* species.

The nature of the pastures found in the highlands also varies a great deal. For Ethiopia, Kahurananga (1981) classifies them into 4 groups:

- The permanent pastures: these are confined to very small areas because of the population pressure.
- Fallow lands: these constitute the main source of grazing. The duration of the fallow varies from 1 to more than 30 years.
- Bottomlands: these are the depressions with heavy clay soils, which are usually very waterlogged during the rains. In the dry season they are very important grazing lands. From these types of lands hay can be obtained.
- Marginal lands: these constitute eroded and overgrazed slopes. They are of limited importance as pastures and are especially found at low elevations (1500 – 2000m).

In these different natural pastures a certain *Trifolium* species may be found being adapted to the specific condition of the pasture. In any case *Trifolium* species are the ubiquitous components of such natural pastures.

2.2 *Trifolium steudneri* Schweinf.

2.2.1 General features

T. steudneri is described as an annual glabrous herb, which can reach up to 60 cm (Thulin, 1983). The leaflets are more or less narrowly elliptical in shape and can have a size of up to 40 mm by 8 mm. Petioles are up to 3 cm long, and the stipules with narrowly triangular tips. The inflorescence is more or less globose, with a flower count of about 10-20, up to 13 mm across and having long peduncle. Bracts are several-nerved forming an involucre. The outer bract is up to 7 mm long which is abruptly and sharply pointed. The inner bracts have an oblong shape and can reach up to 3 mm. The calyx is again several-nerved which are up to 40 in number and the triangular lobes are 2-3 mm long. The corolla is purplish in color and can be 7-8 mm long. The pod contains up to 4 seeds.

2.2.2 Ecology

The species is very common in the central highlands of Ethiopia with the altitude range of 1100-2800m asl. It is also reported by Eeghen (1984) that the species can grow at elevations of 3500m. The species is adapted to upland grasslands and bushlands, especially in damp places (Thulin, 1983). It is also reported by Weise (1989), that the species naturally occurs on Vertisols and appears to tolerate waterlogging. The mentioned ecological distribution of the species corresponds with the zone that has the greatest agricultural potential in Ethiopia (Gryseels and Anderson, 1983), which accounts for one quarter of all

the cropped soils in the Ethiopian highlands, thereby increasing the opportunities for a rapid integration of this clover into the cropping system.

Due to the broad altitude distribution, *T. steudneri* is exposed to a wide range of temperature, radiation and rainfall. Weise (1989) in his study to investigate the effect of temperature on the growth of *T. steudneri,* reported that an increase in the mean shoot (air) temperature (between 15 and 21^{0}C) markedly increased the mean relative growth rate.

The highland areas have an annual rainfall of 500-2000 mm and this is, of course, a very important factor contributing to the moisture condition of the soil. However, the relative position in the field is also important: in a depression or near a riverbank, a plant will have access to more water than a plant growing on a slope under the same climatic conditions. Other essential factors are the distribution of the rain and the longevity of the plant. For an annual, like *T. steudneri*, which can complete its life cycle in a few months, total annual rainfall does not have to be very high as long as it is concentrated in a short period of time. Apart from this *T. steudneri* is adapted to rather wet conditions, growing in bottomlands, seasonally waterlogged areas, on the edge of permanent swamps and near river banks (Eeghen, 1984).

2.2.3 Breeding system and ploidy level

Pritchard and Mannetje (1967), found out that most annual *Trifolium* species that they studied including *T. steudneri* are autogamous. Bees are often involved in the pollination of the plants. Pritchard and Mannetje (1967) found out that one annual and three perennial clovers out of 12 were out-crossers while the remaining eight annuals were all self-fertile.

Among those out-crossing were *T. africanum* var. *africanum*, *T. burchellianum*, *T. semipilosum* and *T. masaiense*. Not all self-fertile African annuals appear to be strictly autogamous but little is known about their degree of out-crossing. Bogdan (1977) reported 1% to 10% out-crossing in only one autogamous annual, *T. rueppellianum*.

Pritchard and Manhetje (1967) observed the cross compatibility of the different species, using *Trifolium masaiense* and *T. semipilosum* as mother plants. Their findings indicate that at least *T. semipilosum*, *T. masaiense*, *T. rueppellianum* var. *rueppellianum* and *T. pseudostriatum* are cross compatible.

Generally, according to Hartl (1980), the selective advantages conferred on highly self-fertilizing species, include the following:

- A high homozygosity for alleles such as those that determine allozymes;
- The proportion of polymorphic loci remains comparable to those in out-crossing species;
- A lower number of deleterious recessive genes than in species that outcross because of elimination of the recessives by natural selection;
- Recombination in naturally selfing species will result in new genetic types not already present in the parent.

These points are of interest in light of both intra- and inter-specific diversity in native clovers.

Most of the African clovers so far studied are diploid (2n=16), including *T. steudneri* (El-Kholy, 1990). It is also reported by Zohary (1972) in genus *Trifolium* an entire aneuploid

series of X = 8, 7, 6, 5 has been encountered, though 8 remains the prevalent number. In the genus *Trifolium*, polyploidy appears to be of little evolutionary importance (Evans, 1967). The majority of clovers are diploids and, as a consequence, are less amenable to widespread inter specific hybridization.

Chromosome sizes of diploid African *Trifolium* species were found to be small and this feature appears to be related to annuality (Pritchard, 1972). Interestingly, the chromosome size reduction, which appears to have occurred in the African genus *Trifolium* is phylogenetically retrograde to the primitive chromosome size condition. Further studies on the chromosome numbers (X=8), growth forms and karyotype in the African annual clovers seem to suggest they are primitive in contradiction to the fact that annuality is supposedly a highly evolutionary specialization.

2.2.4 Photoperiodism and temperature

Pritchard and Mannetje (1967) and Mannetje and Pritchard (1974) reported temperature and photoperiod interaction to affect flowering of some African clover species. Consequently, a clear line between short day and long day plants is difficult to draw. *T. steudneri, T. rueppellianum* and *T. masaiense* were less affected by temperature and day length. They are thought to be photoperiodically neutral (Bogdan, 1977).

In the study of pollen viability, germination and growth carried out by Pritchard and Mannetje (1967), pollen germination was best at 25^0C. In the similar experiment, photoperiodism of ten East African *Trifolium* species was investigated, which includes *T. steudneri*. It was found that the species flowered when the day length was between 10 and

11 hours. In another trial, both day length (10, 12 and 14 hours) and night temperature (20^0C and 10^0C; day temperature was 25^0C for both treatments) were varied. In this trial *T. steudneri* was either day-neutral or could not clearly be classified because of the strong effect of night temperature on flowering.

Temperature alone on the African clovers is probably important as a regulatory rather than as a selective force. High temperature in the tropics is frequently associated with moisture deficits, which may affect the plants' physiology. Water stress, on the other hand, is a significantly selective force although there is also little evidence that it has any direct regulatory action on flowering initiation in plants (Murfet, 1977).

2.2.5 Symbiosis with *Rhizobium* strains and nitrogen fixation

In their natural environment, the *Trifolium* species nodulate freely and fix nitrogen effectively. Norris and Mannetje (1964) studied the symbiotic specialization of several *Trifolium* species from East Africa. These were first screened against 24 *Rhizobium* isolates obtained from African *Trifolium* species, after which the effectiveness of the best inocculant strains for each species was assessed.

In the screening process, an extreme degree of symbiotic specialization within this group of plants was observed. *Rhizobium* strains that are effective with one species are ineffective, or may not even nodulate with the second species; a strain that is effective on one variety may be ineffective on a second variety; finally, the reaction of a number of introductions of the same species to the same range of *Rhizobium* strains may be quite different. The results

stress the importance of correct identification of a species or variety in order to assure effective inoculation (Norris and Mannetje, 1964).

On the grounds of the results of the effectiveness trial, Norris and Mannetje (1964) distinguished two *Rhizobium* affinity groups among the East African *Trifolium* species. Members of each group showed effective symbiosis with their own and each other's *Rhizobium* isolates, but not with isolates from members of the other group.

The two groups consisted of the following species and varieties:

Group one: *T. rueppellianum, T. tembense, T. usambarense, T. baccarinii, T. steudneri, T. burchellianum var. johnstonii*, and *T. pseudostriatum*.

Group two: *T. semipilosum var. semipilosum, T. masaiense* and *T. cheranaiense*.

It has been reported that a range of 70 to 90% nitrogen fixation was observed for many forage legumes (Heichel *et al.*, 1983; Ayoub, 1986). In the sward growth trial of Weise (1989), *T. steudneri* fixed about 61 kg nitrogen per hectare at Addis Ababa in both 1985 and 1986 growth seasons and 80 kg nitrogen per hectare at Debre Zeit in 1985 and 165 kg per hectare in 1986. The primary determinant of these differences was the variation in the dry matter yield. Heichel *et al.* (1983) reported between 70 and 160 kg fixed nitrogen per hectare for the seeding year of several temperate legumes and Ayoub (1986) cited 60 to 120 kg fixed nitrogen per hectare for several tropical legumes. It can therefore be concluded that under appropriate temperature and soil aeration conditions, *T. steudneri* is a very good fixer of atmospheric nitrogen in symbiosis with the indigenous *Rhizobium* population. At the

same time, the species can grow and fix nitrogen even under severe waterlogging conditions, although the nitrogen yield may be low due to poorer growth.

2.2.6 Fodder value of *Trifolium steudneri*

The potential value as fodder plants of several of the East African *Trifolium* species has been assessed at different places and occasions. The attention given to the different species is very unevenly distributed. Most of the work has been done on *T. semipilosum*, which was taken into cultivation as early as 1965. For the other species, including *T. steudneri*, information is sporadic and sometimes non-existent (Eeghen, 1984).

2.2.7 Growth and appearance of the plant

The plant has an erect type of growth with stolons and creeping-ascending side branches. The erect growth and the sparse leaves are common appearances of the species. The erect growth habit can make it possible for this species to be cultivated in a mixed pasture with suitable grass species. The species shows a rapid early growth (Britten, 1962), and can easily be grown from the seed.

2.2.8 Yield

Many of the publications on East African clovers are descriptive, and few data on yields could be found. Production figures of clovers grown in pure stand are particularly scarce (Eeghen, 1984).

Britten (1962), estimated yield by measuring the volume of the plants. Using this method, he found that the yield of *T. steudneri* to be greater than that of *T. tembense* and *T. semipilosum*. Kahurananga (1982) reported at the ILCA headquarters, in the highlands of Ethiopia, that the yield obtained from *T. steudneri* is about 2.2 tons per hectare dry matter yield. In his study of response to phosphorus fertilizer, Akundabweni (1984a) reported that

there was a yield increase from 0.5 to 4.3 tons per hectare dry matter yield on increase of fertilization from 0 to 30 kg per hectare. In this trial *T. steudneri* was grown in a mixture with grasses.

In the study of Weise (1989), the above ground dry matter yield of *T. steudneri* was 1.7 times greater at Debre Zeit than at Addis Ababa in 1985 and 3.7 times greater in 1986. In addition, there was a 2.4 times higher yield at Debre Zeit in 1986 when compared to 1985. The yield at Addis Ababa in 1986 in this trial was lower than the one in the sward growth trial in the same year (252g versus 346g per meter square) probably due to more severe waterlogging caused by a depression in the field of trial. The 9.1 ton per hectare yield after 104 days of growth at Debre Zeit in 1986 compares favorably with the 4 to 6 ton per hectare average report for several tropical (Ayoub, 1986) and temperate forage legumes (Heichel *et al.*, 1983) for the seeding year. The result also shows that *T. steudneri* swards were able to produce between 2.3 and 3.8-ton above-ground dry matter per hectare even when they were exposed to between 7 and 12 weeks of inadequate soil aeration. This clover thus appears to tolerate extended periods of waterlogging, although it grows distinctly better under non-waterlogged conditions, as was the case at Debre Zeit in 1986 with an average soil oxygen partial pressure of about 13kPa.

2.2.9 Nutritional value

According to Eeghen (1984; cited Dougali, 1962), the percentage of crude protein and crude fiber in the dry matter are given for *T. steudneri* to be 19% and 20% at full flowering. Percent dry matter digestibility has been measured for *T. steudneri* by Kahurananga (1982) to be 74%.

The seed yield of *T. steudneri* is reported to be 815 kg per hectare by Akundabweni (1984a), which is one of the highest yield value from the East African clover species.

Frothy bloat, which is due to the development of stable foam within the rumen content, can develop at any time of the year and on any type of pasture, but tends to be more common in the spring and autumn on clover-dominated pastures. Sudden death can occur when increasing ruminal pressure results in circulatory and respiratory failures (Familton, 1990). Frothy bloat is dietary in origin and occurs particularly in cattle grazing pasture with high legume content. A number of contributing causes have been identified, and in most cases a combination of these causes is responsible for the development of the condition. The most important factor appears to be the presence within some legumes of a primary foaming agent (uncondensed tannin) in the leaf cytoplasmic protein. Uncondensed tannin forms a foaming complex with the active cytoplasmic protein. Saponin is also a chemical which is found widely in plants that have a detergent properties and form lather, causing a bloat. Another factor is the rapid rate at which bloat-producing plant material is broken down by the rumen microflora, thus increasing the rate of gas production and protein release. Salivary constituents along with plant lipids appear to play an important role as anti-foaming agents within the rumen (Wheeler, 1987; Familton, 1990).

With regard to disease and pests of this species, only few observations have been done. Norris and Mannetje (1964), carried out a plot experiment in order to test nematode susceptibility. *T. steudneri* showed susceptibility to root knot nematodes. But the infection did not kill any of the plants.

It was mentioned by Eeghen (1984) that except *T. semipilosum*, all species of the East African *Trifolium* genus are undomesticated. This shows that there are still many possibilities to improve the material by selection and breeding.

2.3 CHARACTERIZATION

Characterization of forage germplasm is an essential prerequisite for use of forage genetic resources. Scientists should know what variation exists in gene bank collections. The grouping of accessions resulting from characterization will facilitate further evaluation and use. Agronomists can select accessions from different groups to test a wide variety of genetic material, or can limit themselves to evaluating accessions from one group with characteristics in which they are interested (Wouw *et al.*, 1999).

For accessions in collections to be of value to breeders, information must be available about the samples and their genetic characteristics. Some of the information consists of data recorded at the time of collection (identification of the accessions and of the location of collection and of the ecological conditions at that location), and any names or accession numbers that have been allocated to the accession by the collectors or curators. Such information is called "passport data".

The function of characterization is the recording of basic botanical characteristics of samples that can be easily seen or easily measured and which have a reasonably high heritability in any environment. Preliminary evaluation includes the recording of a limited number of additional traits agreed by a consensus of users of the particular crop to be useful for plant breeding or agronomy. These traits also include those that can be easily seen or otherwise easily measured. There is no clear line of demarcation between the two functions, according to Williams (1984).

2.3.1 Characterization using morphological markers

Morphological characters have traditionally been used as a basis for classification since early days of taxonomy and overwhelming reliance is still placed on morphological traits to produce practical classifications. Practical consideration so far demanded that any genetic constituent be expressed morphologically for identification and characterization of plant germplasm. In genebanks, studies of morphology form part of the characterization work. There are two sets of data generated in genebanks: Characterization and evaluation data. Characterization includes those traits that are highly heritable, can easily be seen and equally expressed in all environments. Furthermore, a limited number of additional traits that are thought desirable can be included in the characterization work. On the other hand, evaluation involves traits that are susceptible to environmental differences but generally useful in crop improvement (Abebe Demissie, 1996).

2.3.2 Characterization using biochemical markers

Plant gene pools are reservoirs of variation, which provide the raw material for crop improvement. Samples in the form of seeds or whole plants, representing the spectrum of genetic variation within cultivated species and their wild relatives, are currently being collected and maintained in genebanks and field genebanks throughout the world. Of fundamental importance in the management of these resources is the measurement and characterization of the variation they represent (Simpson and Withers, 1986).

Morphological and agronomic evaluation of accession variability may be supplemented by a direct study of the genome by isozyme electrophoresis. Several texts illustrate the usefulness of this technique in plant breeding and other applied areas, as well as in more basic studies of genetics and developmental biology.

2.3.3 Evaluation of genetic resources

Numerous morphological data are difficult to comprehend in terms of pattern of variation among accessions. For this reason, a valuable extension of the morphological approach is the use of numerical taxonomic techniques to simplify these complex patterns. The results are diagrammatic summaries of the variations within collections, which allow breeders more easily to choose a specific plant type from the range available, or select dissimilar types for initial crosses to generate the widest range of recombinant genotypes. Numerical taxonomic evaluation can also be related to the original source of the material, identifying geographical regions in which diversity is at a maximum, and therefore guiding future collection programs. Although such phenotypic evaluation will always be important, the data are not easily understood at the gene level, since most characters of interest are

polygenically inherited and are the result of interactions between genotype and environment.

Useful supplementary information can be obtained by the use of biochemical methods. For example, genotype may be distinguished by comparing chromatographic separations of their flavonols or anthocyanins, but again, spots on the chromatogram are not readily analyzed in terms of the genes, which encode them.

It is here that isozyme electrophoresis can yield more direct genetic information, such as markers, which may be used to identify particular genotypes thereby facilitating removal of duplicate samples from genebanks. The level of genetic diversity within and among accessions can be estimated and changes in this diversity during storage can be monitored. Outside the genebank, isozymes can provide data on the genetic relationships within and between natural populations and indicate the most efficient approach to future sampling.

2.4 ISOZYME ELECTROPHORESIS

As cited in Scandalios (1974) and Simpson and Withers (1986), the term 'isozyme' (synonymous with 'isoenzyme') was mentioned to have been proposed by Markert and Moller in 1959, for multiple forms of an enzyme sharing a catalytic activity, derived from a tissue of a single organism.

By placing tissue extracts in a gel medium such as starch or polyacrylamide and applying an electric current, protein molecules migrate at a rate determined by their net charge and the pH of the medium. In addition, the network of gel molecules sieves migrating molecules according to their size and shape. Active enzymes can be separated thus into discrete bands and their position made visible by the use of specific enzyme stains.

Since the amino acid sequences of proteins are determined by nucleotide sequences of coding structural gene loci, "the analysis of a protein structure using electrophoresis is, to a first approximation, an analysis of a gene". Gene mutations, which cause substitution, deletion or addition of amino acids within the structure of an enzyme, may be detected if these changes affect electrophoretic migration. The different molecular forms of an enzyme are called 'allozymes' if their polypeptides are coded by different alleles at a single locus. The term 'isozyme' may be reserved for enzyme forms, which catalyze the same reaction but are coded by more than one gene locus, although the word tends to be used more generally to denote both allozymes and isozyme in the narrow sense.

Allozyme band patterns are usually controlled by codominant alleles and inherited according to monogenic Mendelian ratios. The number of polymorphic bands on a

zymogram is dependent on the number of loci, the number of alleles per locus and the quaternary structures of the enzyme.

A major advantage of electrophoresis over morphological evaluation is the speed with which analyses can be conducted on a large number of individual plants, often as seeds or seedlings, without the need for field experiments. One potential application in genebanks is for the identification of accessions, but the usefulness of the technique depends on the amount of genetic variation within and between test samples.

The power of the isozyme technique extends far beyond identification of genotypes. It is a method for measuring genetic variations close to the DNA level, relatively free of environmental effects, and on a manageable number of samples.

The analysis of isozyme data can provide estimates of genetic variation within natural populations in terms of the proportion of loci that have more than a single allele, the number of alleles per locus, and the mean proportion of genes, which are heterozygous per individual. The degree of genetic similarity between different populations can be assessed by comparing the frequencies of alleles they have in common at particular loci.

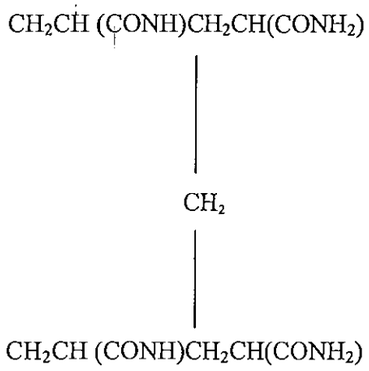
In general, isozymes are interesting genetic markers for the study of genetic diversity. The use of isozyme markers with their more frequent occurrences in plant materials that compensate for some of the drawbacks of the morphological markers, enable the localization of several loci responsible for quantitative traits (QTLs). Isozymes are one of the popular marker systems used in population studies. Unlike low molecular weight

markers such as flavonoids or phenolics, the clear genetic basis of isozyme variability is one of the reasons for their wide utilization in population characterization. Although the characterization and identification of isozymes is carried out on the basis of the phenotypic electrophoretic band patterns, these patterns can usually be interpreted in terms of loci and alleles. These qualities make isozymes one of the most ideal marker systems in population studies especially in assessing variation within and among populations. In addition to these properties, isozymes have many desirable advantages. These include: **a)** allelic expression is usually codominant, free of epistatic reactions and usually unaffected by environmental conditions, **b)** allelic differences are always reflected in terms of mobility differences, **c)** enzyme systems to be considered are usually selected for technical reasons irrespective of their level of polymorphism, thus implying random sample of the genome. Like many other marker systems isozymes have some shortcomings. Not all genetic changes occurring at DNA level are detected at protein (isozyme) level. Moreover, lack of representativeness was ascribed as one of the drawbacks since one set of structural genes are only represented in these types of proteins. An important limiting factor of isozyme systems in population studies is the relatively low number, which can actually be detected by gel electrophoresis and specific staining. Furthermore, polyploidy is quite a common phenomenon in plants and caution is required when interpreting patterns resulting from gene duplication in polyploid species. Cryptic variations associated with the fact that different enzymes show the same electrophoretic mobility was ascribed as one of the shortcomings of isozyme markers (Abebe Demissie, 1996).

2.4.1 Acrylamide gel

Electrophoretic separation of complex mixtures of proteins can be accomplished in several types of support media, including starch, polyacrylamide, and agarose gels, and cellulose acetate membranes. The latter two types typically lack sufficient resolving power. For this and other reasons they are generally not employed for the study of enzyme polymorphism. Biochemists and others requiring maximum resolving power often prefer polyacrylamide gel electrophoresis (PAGE). A valuable property of PAGE is that the stringency of molecular sieving can be varied by altering the acrylamide concentration, thus increasing flexibility in the range of protein molecular weights or dimensions that are separable. Other advantages of polyacrylamide gels include their uniformity and transparency, facilitating densitometric quantification of product, inertness of components allowing broad assay compatibility, usually rapid run times, and the ability to concentrate proteins into very thin starting zones in stacking gels prior to their entrance into the resolving gels. Some enzymes, such as ribonuclease, pyruvate decarboxylase, and amylase are analyzed with PAGE because the assays involve production of white precipitate. Disadvantages of polyacrylamide gels compared to starch gels include the neurotoxicity of the acrylamide monomers used for PAGE, complexity of gel preparation, high costs of equipment, difficulty in sample loading and the need of centrifuged samples. Probably the most compelling reason is the amount of data generated per gel is less for polyacrylamide gel compared to starch gel. This is because starch gels can be sliced, allowing multiple (up to six), enzyme systems to be assayed per gel, whereas polyacrylamide gels are usually stained for a single enzyme (Wendel and Weeden, 1989).

Acrylamide possesses the advantages of the 'molecular sieving' effects of starch gels while providing ease of preparation and handling, gel clarity, and for some protein systems, improving resolution and increased reproducibility. The chemical structure of the gel is a three dimensional linear polymer of acrylamide cross-linked by methylene bridges:



Except for the terminal portions of the acrylamide polymer the gel media is free of hydroxyl and acetic groups, providing a more inert support media than starch. The range of pore sizes and the compatibility of a wide variety of buffers with polyacrylamide gel provide versatility equal to that of starch support media.

2.5 CHARACTERIZATION DATA AND SPATIAL ANALYSIS

Genetic resources management is a complex process that includes a number of mutually dependent stages, from the identification of a target gene pool for conservation to the use of genetic resources. Many of these activities not only generate but also require geo-referenced data (having geographic co-ordinates). The management and analysis of these data, including together with geo-referenced data from other sources, are crucial to the effectiveness of the process of plant genetic resource management activities. To add

significantly to the value of genetic resources, Geographic Information System (GIS) are becoming increasingly important in the management of large and complex datasets, which are associated with plant genetic resources (Guarino *et al.*, 2001).

Components in this process generate various types of data, including information on the identity (passport data) and characteristics of germplasm (characterization and evaluation data). These data mostly refer to stands of wild or cultivated plants found growing in specific, known locations on the surface of the earth. These locations are also included in the passport data of gene bank and herbarium documentation systems. Those data associated with germplasm are geo-referenced and therefore amenable to the description and modeling of patterns and relationships in geographical data (Bailey 1994), which is referred to as spatial analysis.

In the process of conservation and use of genetic resources, we do not only generate but also require data. If we take germplasm collection, this process results in data on the distribution, the phenology, the ethnobotany and, finally after characterization and evaluation work, the genetic diversity of the target taxon. Such data acquired in previous collection missions can be found in gene bank and herbaria documentation systems and also in the literature. Therefore, a collector benefits from considering, before venturing out into the field, any such data that may already exist (Hijmans *et al.*, 1999; Hijmans *et al.*, 2001; Guarino *et al.*, 2002).

Such data, which are geo-referenced, coming out of the genetic resources management process can be analyzed not only on their own, but also together with other geo- referenced

data from additional sources. Insufficient data on previous collections may force a collector to use additional sources like climatic, vegetation and soil data from the study region to estimate the distribution and phenology of the taxon under consideration. As knowing the scientific name of a plant opens a door into the taxonomic, ecological and ethnobotanical literature, and all the information about the taxon, knowing the localities of sites where the plant grows allows access to the rich variety of geo-referenced environmental and socio-economic data available in maps and digital forms (Guarino *et al.*, 2002).

2.6 ROLE OF CORE COLLECTION IN CHARACTERIZATION AND EVALUATION STUDIES

A core collection consists of a limited set of accessions derived from existing germplasm collections, chosen to represent the genetic spectrum in the whole collection. The core should include as much as possible of the collections genetic diversity. The remaining accessions in the collection are called reserve collections. This implies that a core should not contain duplicates and should minimize similarity between its entries (Brown, 1995).

When we look at the functions of core collections, they have many roles to play in the management and use of genetic resources. This is because most activities in gene bank management require the curator to make choices or to prioritize amongst accessions because of limited resources. It is usually not logical or efficient to start at accession number one and work sequentially through all accessions in the collection. According to Brown (1995), characterization and evaluation are germplasm operations for which a core collection offers distinct advantages.

In characterization, a core includes suitable material for developing an adequate list of descriptors. A sufficient number of characters and states should be used to distinguish between its entries. In evaluation work, a core enables a logical and efficient two-step procedure to be carried out in sampling the whole collection. Its entries can be the first to be evaluated for expensive or complex traits. Further, by focusing evaluation on a restricted set of accessions, the core assists the development of a multivariate database to study the interrelationship between characters and between kinds of data (passport, characterization and evaluation of characters) (Brown, 1995).

3. MATERIAL AND METHODS

3.1 MORPHOLOGICAL STUDY

3.1.1 Description of study area

The experiment was conducted at ILRI-Debre Zeit research station. It is located on 8 ^{0} 44 'N latitude and 38^0 58'E longitude at the elevation of 1850 m asl. The eco-climatic zone of the area is sub-tropical grassland with a light clay-loam soil type (30% clay, 35% silt, 34% sand, 5% loam). The soil pH is about 6.7 with total organic matter of 5%, which is fairly high and available Phosphorous of 4.8 ppm, which is very low. Average annual rainfall of the research area was reported to be 910.7 mm, with an average annual maximum temperature of 25.5^{0}C and average annual minimum temperature of 12.3^{0}C (Akundabweni, 1984a; Kamara and Haque, 1988 and Tesfayesus Seiro, 1992). The detail for rainfall and temperature of the area is given in Appendix 10 (A). For the year 2001, rainfall and temperature data are also given in Appendix 10 (B).

3.1.2 Experimental design, treatments and number of replicates

In this study 50 accessions of *T. steudneri* collected from different regions and altitude ranges were used. The list of accessions is given in Appendix 1. The germplasm collections were acquired from ILRI, Forage Genetic Resource project, Addis Ababa, Ethiopia. Even though accessions were picked randomly from the 85 collections available, the selected accessions were checked as much as possible to be representative of the highlands, using DIVA-GIS software map output.

A fertility gradient was expected down the field slope. Three blocks (replications), each with 3m by 27m plot size were used. In each block all the 50 accessions (treatments) were represented so that each accession was represented three times in the experiment. Accessions were sown in a single row (3m length and 0.5m between rows), being located randomly using a random number. Therefore each block has its own random location of accessions. In general this was a randomized complete block design with three replications (blocks).

The information in the third and fourth column of Table 1 is taken from the passport data, which were recorded during the germplasm collection period. The regional information in this passport data was based on the old regional classification. In the fifth column the new regional classification is given.

3.1.3 Experimental Protocol

Depending on the time of flowering, data collection was made 10-12 weeks after planting. In taking data, a standard protocol was referred for the morphological study from a descriptor for White clover (IBPGR, 1987; IBPGR, 1992). Fifteen plants per accession were chosen randomly and the data were scored for all the traits that can be taken at the same time from those plants.

3.1.3.1 Quantitative characters

1) Stem thickness (**stmthk**): at 50% flowering (of the accession), using a caliper (mm), the sixth internode from the bottom of the measured section (primary stolon) was considered.

2) Internode length (**indlgt**): at 50% flowering, by measuring the length of the stolon divided by the number of nodes on it.

3) Leaflet length (**leflgt**): at 50% flowering, all leaflet measurements were made on the fourth leaf from the distal end of the primary stolon (the tallest part of the plant). Using a ruler (mm), measurement was made at the middle leaflet from base to tip.

4) Leaflet width (**lefwdt**): by taking the same leaflet, the widest part was measured using a ruler (mm).

5) Petiole length (**petlgt**): taking the same leaf, length of the leaf stalk was measured using a ruler (mm).

6) Stipule length (**stplgt**): taking the same leaf stalk, the stipule was measured from base to tip using a ruler (mm).

7) Stipule width (**stpwdt**): the widest part of the stipule was measured using a ruler (mm).

8) Flower length (**flwlgt**): all measurements of flowers were made on a fully expanded flower (head) from the tallest part of the plant. Length of the outer-most petal (embracing the other petals) of the middle floret (of the head) was measured using a ruler (mm).

9) Flower width (**flwwdt**): taking the same petal, the widest part was measured using a ruler (mm).

10) Flower number (**flwnum**): counting the number of florets per head.

11) Peduncle length (**pedlgt**): taking the same flower from which we measured other flower characters, the flower stalk was measured using a ruler (mm).

12) Number of seeds per pod (**sedpod**): counting the number of seeds from a mature pod.

13) 100-seed weight (**hunswt**): by measuring the weight (gm) of 100 seeds after threshing.

14) Calyx nerve number (**clxnum**): counting the number of nerves for the calyx found exactly under the outer most petals, using 10X-magnifying lens.

3.1.3.2 Qualitative characters

1) Growth habit (**flwcol**): at 50% flowering, by observing whether the plant is decumbent, semi-erect, semi-erect and creeping-ascending or creeping-ascending.

2) Stem hair (**stmhar**): at 50% flowering observed (if needed using 10X magnifier) whether the stem surface is smooth, sparse soft hairs, medium dense soft hairs or dense soft hairs.

3) Leaflet shape (**lefshp**): at 50% flowering, by observing whether the shape of the leaflet is linear, oblong or linear and pointed.

4) Upper leaflet surface hair (**uplfha**): at 50% flowering (if needed using 10X magnifier) by observing whether the upper surface is smooth, sparse soft hairs, medium dense soft hairs or dense soft hairs.

5) Lower leaflet surface hair (**lwlfha**): at 50% flowering (if needed using 10X magnifier) by observing whether the upper surface is smooth, sparse soft hairs, medium dense soft hairs or dense soft hairs.

6) Leaflet mark (**lfmark**): at 50% flowering, by observing whether there is a mark on the leaflet tip, leaflet base or no leaflet mark.

7) Leaflet nerve color (**nercol**): at 50% flowering, by observing whether the leaf nerve is lighter or darker.

8) Flower color (**flwcol**): from the fully expanded head, taking the outer most petal of the middle floret and observing the color by using Royal Horticultural Society (RHS) color codes.

9) Calyx lobe shape (**clxshp**): from the fully expanded head, taking the middle floret to observe whether the shape is aristate, circular or oblong.

3.1.3.3 Agronomic characters

1) Days to 50% flowering (**flow50**): by counting the number of days when half of the plants within a row (accession) start flowering.

2) Days to 75% maturity (**matu75**): by counting the number of days when 75% of the plants within a row are matured (100% flowering).

3) Plant height (**plthgt**): at 50% flowering, measurement was made from the base of the plant to the top of the canopy using a ruler (cm).

4) Leaf stem ratio (**lfstra**): from fresh stands of plants, the ratio will be estimated.

5) Plant weight (**plntwt**): at 50% flowering of rows (which is considered to be the right time to use as forage), randomly chosen plant was cut and fresh weighed.

6) Dry matter (**drymat**): at 50% flowering of rows (which is considered to be the right time to use as a forage), a randomly chosen plant was cut and air dried for three weeks and weighed to determine the dry matter.

3.2 ISOZYME STUDY

For the isozyme study most of the procedures were taken from Hussain *et al.* (1988) Wendel and Weeden (1989), and Chamberlain (1998). The isozyme analysis was carried out on nine accessions based on the morphological quantitative characters clustering. The accessions were selected from the six clusters and based on the number of members in each group, three clusters were represented by two members and three clusters by one member. Five seedlings per accession were studied. To break dormancy of the seeds, the plated seeds were left in the cold room for a week. Then the plates were transferred to an incubator for germination. This was a procedure followed at ILRI-Addis Forage gene bank to germinate most *Trifolium* species on a plate. The best resolution was gained from seedlings at the age

of six days. Extraction was made by using whole seedlings. Using a mortar, seedlings were crushed using the following extraction buffer:

- pH 8.3 Tris-HCl 0.05M containing

Sucrose	20%
PVP (Polyvinyl-Pyrrlidone)	5%
Triton	0.5%
2-Mercaptoethanol	14mM

Crushing was made over ice and the crude extract was centrifuged in the cold room at 12000 rpm for ten minutes. The supernatant was removed ready for use. Most of the work was done on ice to reduce damage to the enzymes.

Polyacrylamide gel electrophoresis (PAGE) was used for the study and the following solutions were prepared for the gel solution.

1. Polyacrylamide solution:

Polyacrylamide	22.2 g
Bisacrylamide	0.6 g

Dissolved in about 60 ml of distilled water, filtered in to 100 ml volumetric flask and the final volume was adjusted to 100 ml mark with water.

2. Buffer solution (suitable for Lithium borate buffer system)

   5.4 Tris-base (pH 8.3)

   1.28 g anhydrous citric acid

   100 ml electrode buffer used for the Lithium-borate buffer system

   900 ml deionised water

3. Persulphate solution:

   0.5 g of ammonium persulphate was dissolved in 5 ml of distilled water. This solution was made fresh each time before use.

4. TEMED (Tetramethylenediamine): with normal concentration.

The above solutions were mixed in the following proportion to make 10% acrylamide gel concentration (running gel).

- Polyacrylamide solution 22.5 ml, gel buffer 12.5 ml, water 15 ml, persulphate 0.5 ml and finally just before pouring the gel, TEMED 15 µl was added.

After the gel polymerized, the stacking gel was poured on the top of the running gel. To prepare a stacking gel, stacking gel buffer was made (Tris-HCl 0.5M pH 6.8) by dissolving 6.3 g Tris in distilled water and by adjusting the pH to 6.8 using 6N HCl. The final volume was brought to 100 ml and pH readjusted to 6.8.

The stacking gel was prepared as follows:

- Polyacrylamide solution 4 ml, stacking gel buffer 7.5 ml, water 18.2 ml, persulphate 0.3 ml and just before use by adding TEMED 15 µl.

The electrode buffer used in Lithium-borate buffer system was prepared by mixing Lithium hydroxide1.2g, 11.9g boric acid and 1 liter deionised water (the pH was adjusted to 8.3). A vertical PAGE was conducted by using 300 volt at 80mA for the stacking gel and 250 volt at 70mA for the running gel for about two hours and half.

Six enzymes were checked during the preliminary survey. These were Peroxidase (PRX: EC 1.11.1.7), α-Esterase (α-EST: EC 3.1.1), β-Esterase (β-EST: EC 3.1.1), Acid phosphatase (ACP: EC 3.1.3.2), Aspartate aminotransferase (AAT: EC 2.6.1.1) and Malic enzyme (ME: EC 1.1.1.40). Out of these systems Peroxidase (PRX), α-Esterase (α-EST), β-Esterase (α-EST) and Acid phosphatase (ACP) were selected for the trial, since they gave consistent results. In this analysis to make the data analysis easy for the software, α-EST was designated as ESA and β-EST as ESB.

The following staining recipes were used after the protocols developed by Hussain *et al.* (1988); Wendel and Weeden (1989); Chamberlain (1998).

For PRX: Buffer/stain solution:

- Sodium acetate 0.2M buffer pH 5.0 5ml
- 3-Amino, 9-Ethyl carbazol 40mg
- Hydrogen peroxide 30% 2 drops

By dissolving Amino ethyl carbazol in 5ml of Dimethyl formamide, the gel was kept floating in acetate buffer/stain and then hydrogen peroxide was added. Incubation was at room temperature, then washed with water and finally fixed in 50% ethanol.

For ESA: Buffer/staining solution containing

- 20ml water
- 20ml Sodium dihydrogen orthophosphate 0.2M

- 10ml disodium hydrogen orthophosphate 0.2M

The substrate is 2ml 1% α-napthyl acetate in acetone and the dye is125mg fast blue RR salt in 1ml acetone. Incubation was made for an hour at 37^0C.

For ESB: Buffer/staining solution containing

- 20ml water
- 20ml Sodium dihydrogen orthophosphate 0.2M
- 10ml disodium hydrogen orthophosphate 0.2M

The substrate is 2ml 1% β-napthyl acetate in acetone and the dye is125mg fast blue RR salt in 1ml acetone. Incubation was made for an hour at 37^0C.

For ACP: The buffer/staining solution

- 50ml 0.4M sodium acetate buffer, pH 5.0 (pre-soaking).
- 50ml 0.2M sodium acetate buffer, pH 5.0

The substrate is 50mg α- napthyl acid phosphate and the dye is 50mg KK fast black K salt and 0.5ml of 10% Magnesium chloride. The gel was socked for 20 minutes at 4^0C in pre-soak buffer and then transferred to stain buffer. Then incubation was made for an hour at 37^0C.

Gels made were scored and photographed just after staining and the variation in banding patterns was determined by the migration from the origin towards the anode. On the zymogram, isozyme bands were designated to define the general area. Only clearly visible loci (bands) were subjected to scoring. This can bring consistency in the scoring activity. Biosys software (Nei, 1978) was used to show the phenotypic polymorphism,

heterozygosity, genetic distance and degree of differentiation ($F_{st.}$). Wendel and Weeden (1989) reported different enzyme structures in plants and based on that banding patterns were genetically interpreted.

3.3 STATISTICAL ANALYSIS

Using SAS (1999) computer program, the data was subjected to analysis of variance (ANOVA). Cluster analysis was done based on the quantitative data using MINITAB (1998) software and the clustering of agronomic characters used the PATN (Belbin, 1988) computer program. Chi-square test was made by the MSTATC (1991) computer program using region and altitude ranges as a classifying variable. Factor analysis and Mahalanobis's distance were carried out using SPSS (1999) software. Allele polymorphism and genetic distance was determined using the Biosys computer program (Nei, 1978; Swofford and Selander, 1981).

Variance components, coefficient of variation, heritability and genetic advance were determined as follows:

Phenotypic variance (Vp)=genotype ms/r; error variance (Ve)=error ms/r, genotypic variance (Vg)=Vp-Ve, where r = number of replications and ms= mean squares.

Phenotypic coefficient of variation (PCV)=100*$\sqrt{}$ (Vp) /m and genotypic coefficient of variation (GCV)=100*$\sqrt{}$ (Vg)/m where m= the mean value. Heritability (h^2)= Vg/Vp .

Genetic advance (GS) = (I)(h^2) $\sqrt{}$ (Vp) where I = selection differential (2.06 for selecting 5% of the genotypes); gs (% of the mean)=(gs/m)* 100 (Belay, 1997).

4. RESULTS

4.1 MORPHOLOGICAL CHARACTERIZATION

4.1.1 Mean values of quantitative characters

Mean values of the 19 quantitative morphological characters by region and altitude group are given in Tables 1 and 2 respectively. The mean values of these characters for each of the 50 *T. steudneri* accessions are given in Appendix 2. Table 3 shows summary of the mean square values of block, treatment and error from the analysis of variance for the 19 morphological quantitative characters.

Table 1 Mean values for 19 morphological quantitative characters by region.

Region	Stmthk	Indlgt	Leflgt	Lefwdt	Stplgt	Stpwdt	Flwlgt	Flwwdt	Flwnum	Pedlgt	Petlgt	Sedpod	Hunswt	Flow50	Matu75	Plthgt	Lfstra	Plntwt	Drymat
Gojam	2.80	13.66	37.46	5.88	6.38	1.65	4.12	1.94	28.81	38.64	11.74	2.60	0.11	98.84	107.62	168.74	0.91	14.26	4.91
Gonder/Wolo	2.90	13.28	37.14	5.75	6.40	1.69	4.23	1.91	29.67	37.15	11.42	2.59	0.10	98.34	106.34	170.15	0.95	17.09	5.46
Kefa	2.82	15.38	39.05	7.05	7.23	1.74	4.19	1.91	29.09	32.98	9.83	1.99	0.10	92.75	101.59	167.37	0.76	13.15	4.89
Shewa	2.81	14.01	39.07	6.97	7.44	1.75	4.13	1.92	29.24	39.66	11.82	2.55	0.10	90.64	99.53	159.81	0.86	13.27	4.51
Welega	2.78	12.73	36.23	6.42	6.94	1.89	3.87	1.77	29.94	34.44	10.27	2.27	0.10	99.17	107.17	148.84	0.80	11.15	4.14

Table 2 Mean values for 19 quantitative morphological characters by altitude group.

Altitude	Stmthk	Indlgt	Leflgt	Lefwdt	Stplgt	Stpwdt	Flwlgt	Flwwdt	Flwnum	Pedlgt	Petlgt	Sedpod	Hunswt	Flow50	Matu75	Plthgt	Lfstra	Plntwt	Drymat
≤ 1850	2.84	13.74	37.72	6.39	6.88	1.75	4.13	1.88	29.51	36.28	10.95	2.39	0.10	95.84	104.19	163.27	0.86	14.35	4.89
1851-2050	2.80	13.49	39.60	6.51	7.07	1.64	4.10	1.96	28.61	36.00	9.77	2.37	0.10	91.73	102.80	151.80	0.76	11.26	3.87
2051-2250	2.85	13.37	38.51	7.00	7.49	1.77	4.21	1.90	29.39	38.25	11.63	2.51	0.10	91.70	100.70	152.90	0.87	14.49	5.23
2251-2450	2.82	13.83	37.80	6.19	6.70	1.72	4.09	1.90	28.57	37.84	10.96	2.51	0.10	97.42	105.56	168.92	0.90	13.99	4.81
≥ 2451	2.80	14.00	37.56	5.63	6.22	1.60	4.14	1.91	28.77	38.79	11.32	2.63	0.11	98.69	106.67	173.30	0.90	14.63	4.59

Table 1 shows that accessions from Gojam have the highest mean value for flower width, seed per pod, hundred seed weight and days to 75% maturity. Accessions from the region also showed higher mean value for days to 50% flowering. They stand next to Gonder/Wolo accessions in plant height, leaf stem ratio, plant weight and dry matter. The Gonder/Wolo accessions had the highest mean values in stem thickness, flower length, plant height, leaf stem ratio, plant weight and dry matter. The Kefa accessions had the highest mean value for internode length and leaf width. These accessions had longer leaves and stipules next to those from the Shewa region. Accessions from the Shewa region had the highest mean values in leaf length, stipule length, peduncle length and petiole length. The Welega accessions had the largest stipule width, flower number and days to 50% flowering. The accessions from Gojam and Gonder/Wolo performed well for forage agronomic characters like dry matter, plant height, plant weight and leaf stem ratio.

Since the Wolo region was represented by only one accession it tends to over estimate the mean value for most of the characters for that region. Therefore we consider that particular accession together with the Gonder accessions, based on its closeness to the collection site.

Considering the five altitude groups (Table 2), the first altitude group (≤ 1850m asl) had the highest mean value in flower number. Higher results were scored for days to 50% flowering, days to 75% maturity and plant height, next to altitude group five and four. The second altitude group (1851-2050m asl) had the highest mean value for leaf length and flower width. But higher results were found in this group for leaf width and stipule

length. This group had the lowest mean values for plant height, leaf stem ratio, plant weight and dry matter, which are important characters for the species as a forage plant. The third altitude group (2051-2250m asl) had the highest results in stem thickness, leaf width, stipule length, stipule width, flower length, petiole length and dry matter. Higher results were also observed for leaf length, flower number, peduncle length, plant weight and leaf stem ratio. Therefore this altitude group shows a higher mean value in most of the characters under study. The fourth and fifth altitude groups (2251-2450m asl and ≥ 2451m asl respectively) representing the higher altitude areas show highest values of means in the forage agronomic characters. Highest values are also found for the fifth group in internode length, peduncle length, seed per pod and hundred seed weight.

Table 3 Mean squares for 19 quantitative morphological traits of 50 *T. steudneri* accessions.

Characters	Mean Sq. for Block	Mean Sq. for Trt.	Mean Sq. for Error	CV
Stmthk	4.764***	0.509***	0.209	16.259
Indlgt	445.585***	60.803***	11.877	24.868
Leflgt	1385.532***	214.164***	25.177	13.116
Lefwdt	56.826***	14.854***	1.111	16.202
Stplgt	59.167***	13.305***	1.238	15.971
Stpwdt	1.527***	0.318***	0.079	16.402
Flwlgt	14.526***	0.651***	0.125	8.561
Flwwdt	0.114	0.139***	0.047	11.312
Flwnum	79.969**	38.934***	12.616	12.211
Pedlgt	2894.32***	383.798***	48.398	18.136
Petlgt	44.044*	115.795***	11.076	28.749
Sedpod	27.057***	3.692***	0.534	29.040
Hunswt	0.004	0.003	0.003	49.597
Flow50	984.633***	1358.269***	9.853	3.3247
Matu75	1190.605***	1108.811***	22.723	4.621
Plthgt	47281.029***	10253.935***	1659.379	24.996
Lfstra	0.074	0.150***	0.040	22.927
Plntwt	3988.514123***	173.120***	9.507	22.620
Drymat	7.430	55.078***	3.549	39.288

P<0.001 ***, P<0.01 **, P<0.05 *, otherwise ns (non significant).

Analysis of variance over the entire quantitative data showed highly significant differences between populations in almost all the characters except hundred seed weight. Blocking was also effective which showed a highly significant difference for most of the characters except in flower width, hundred seed weight, leaf stem ratio and dry matter.

4.1.2 Correlation Analysis of Quantitative Characters

Association of traits is an important indicator for common elements of genetic control and similar response of characters to selection pressure (Thorpe, 1976). The highly significant correlation between the different characters also indicates the presence of some common elements of genetic control such as pleiotropy and high linkage between genes.

In this study a higher positive correlation was observed between days to 50% flowering and days to 75% maturity, leaf width and stipule length, and plant weight and dry matter (Table 4). Similarly, positive correlation was also found to be high between internode length and plant height, leaf width and stipule width, leaf length and peduncle length, and stipule length and stipule width.

Significantly higher negative correlation was found between leaf length and days to 50% flowering, leaf length and days to 75% maturity, stipule length and days to 50%flowering, and peduncle length and days to 50% flowering. Negative correlation was also high between leaf width and days to 50% flowering, peduncle length and days to 75% maturity, leaf width and days to 75% maturity, and stipule length and days to 75 %maturity.

Table 4 Correlation among 19 different characters of 50 *T. steudneri* accessions.

Characters	Stmthk	Indlgt	Leflgt	Lefwdt	Stplgt	Stpwdt	Flwlgt	Flwwdt	Flwnum	Pedlgt	Petlgt	Sedpod	Hunswt	Flow50	Matu75	Plthgt	Lfstra	Plntwt	Drymat
Stmthk	1.000	-0.142	.366**	0.114	0.056	-0.094	-0.052	-0.103	0.008	0.187	-0.115	-0.012	-0.087	-0.260	-0.230	-0.144	-0.011	-0.004	-0.10
Indlgt		1.000	.417**	.384**	.415**	0.251	0.105	-0.048	0.246	.403**	0.030	-0.046	0.148	-0.225	-0.230	0.760**	0.050	0.284*	-0.166
Leflgt			1.000	.461**	.572**	0.032	-0.219	-0.207	-0.066	0.639	0.106	0.103	0.117	-0.771**	-0.708**	-0.054	-0.057	-0.411	-0.448
Lefwdt				1.000	.889**	.645**	0.004	0.053	.386**	.437**	.463**	0.176	-0.084	-0.626**	-0.606**	-0.048	-0.116	-0.329	-0.106
Stplgt					1.000	.573**	-0.053	0.002	.336*	.487**	.412**	0.151	-0.042	-0.635**	-0.579**	-0.007	-0.064	-0.281*	-0.113
Stpwdt						1.000	0.077	0.144	.445**	0.121	.406**	0.038	-0.185	-0.112	-0.120	0.105	0.060	0.040	0.226
Flwlgt							1.000	.371**	0.122	-0.229	-0.064	-0.019	-0.030	0.251	0.197	0.173	0.184	0.235	0.350*
Flwwdt								1.000	0.186	0.052	.330*	0.010	-0.035	0.269	0.258	0.130	0.183	0.162	0.223
Flwnum									1.000	0.172	0.159	-0.107	0.008	0.060	0.055	0.265	0.087	-0.115	0.017
Pedlgt										1.000	.294*	.321*	0.216	-0.635**	-0.626**	0.086	0.062	-0.358	-0.333*
Petlgt											1.000	0.135	0.039	-0.207	-0.182	-0.059	0.334*	0.070	0.155
Sedpod												1.000	0.273	-0.192	-0.229	-0.099	0.280*	-0.040	0.017
Hunswt													1.000	-0.013	-0.020	0.039	0.057	0.007	0.034
Flow50														1.000	0.968**	0.350*	0.169	0.479**	0.416**
Matu75															1.000	0.340*	0.159	0.490**	0.438**
Plthgt																1.000	0.225	0.055	0.091
Lfstra																	1.000	.287*	0.324*
Plntwt																		1.000	0.839*
Drymat																			1.000

4.1.3 Cluster Analysis

Morphological clustering of the 50 *T. steudneri* accessions was made using the 19 quantitative morphological characters. As shown in Figure 1, the dendrogram, obtained from MINITAB (1998) hierarchical cluster analysis, grouped the 50 accessions into six clusters. Similar clustering result was obtained using PATN hierarchical cluster analysis (Belbin, 1988). The mean of the characters in each cluster are given in Appendix 3.

Kruskal-Wallis values were calculated using PATN (Belbin, 1988) for the 19 morphological quantitative characters (Appendix 5). A summary statistics for these characters is also given in Appendix 4. Kruskal-Wallis test is a non-parametric test (distribution free) used to compare three or more independent groups of sampled data. Kruskal-Wallis values are useful to indicate characters, which contributed high for the differentiation between clusters. The higher the value for a character, the higher the contribution will be. Characters like plant height and internode length showed higher Kruskal-Wallis value.

Cluster I consists of ten accessions, where eight are from Shewa and two are from Gojam. The altitude range for this cluster was from 1780 to 2450 m asl. Taking the five altitude groups used in this study, four accessions belong to the first group (≤ 1850 m asl), one to the second (1851-2050 m asl), three to the third (2051-2250 m asl), two to the fourth (2251-2450 m asl) and none to the fifth altitude group (≥ 2451 m asl).

Cluster II consists of ten members, where five are from Shewa, two from Kefa, one from

Gojam, one from Welega and another one from Wolo. The altitude range for this cluster is from1820-2900 m asl, which is the widest compared to other clusters. Looking at their distribution in the altitude groups, one accession belongs to the first group, one to the second, four to the third, two to the fourth and the others to the fifth group.

Cluster III consists of seven members, where four of them came from Shewa, two from Kefa and one from Welega. The altitude range for this cluster is from 1750-2370 m asl. When we look at their distribution in the altitude groups, two accessions belong to the first, two to the second, one to the third, two to the fourth and no accession was found to belong the fifth group.

Cluster IV consists of three members, where all of them came from Gojam. They were found to have a minimum range of altitude, from 2500-2520 m asl. The three members of this cluster belong to the fifth altitude group.

Cluster V is the largest of all, having 19 members in it. When we look at the regional distribution, nine are from Gojam, eight from Shewa and two from Gonder. The altitude range for this cluster is from 1870-2880 m asl, which is the second widest range next to cluster two. When we look at their distribution in the altitude groups, none of them belong to the first group, one accession belongs to the second, two to the third, nine to the forth and seven members of this cluster belong to the fifth group.

Cluster VI is unique in having one member form Gojam, accession number 9452. This

accession came from an altitude of 1850 m asl, which makes it belong to the first altitude group.

The agronomic clustering was made to assess the productivity of the 50 accessions of *T. steudneri* by taking eight characters, six agronomic and two quantitative morphological characters (leaflet length and width), which are thought to be important for the forage agronomy aspect of the species. Three major clusters were extracted, which can be further analyzed to assess the most productive group. Figure 2 shows the dendrogram from the PATN computer program. In addition to the dendrogram the other output of this program includes a summary of statistics for the three clusters on the agronomic characters measured and Kruskal-Wallis values, together with the probability values. The values obtained are given in Appendix 6 and 7.

Cluster A of the agronomic clustering consists of nine accessions. The altitude ranges from 1780-2380 m asl. This cluster matches with the cluster I of the morphological clustering, regarding their members, except for the absence of one accession, 7658. Eight of these accessions came from Shewa and one member from Gojam. Considering the five altitude groups, four accessions belong to the first group (≤ 1850 m asl), one to the second (1851-2050 m asl), three to the third (2051-2250 m asl), one to the fourth (2251-2450 m asl) and none to the fifth altitude group (≥ 2451 m asl).

Cluster B is the largest group, which has 33 members. Out of these, fourteen are from Shewa, twelve from Gojam, three from Kefa, two from Welega and two from Gonder. Of

these accessions two belong to the first altitude group, four to the second, seven to the third, twelve to the fourth group and the rest eight to the fifth altitude group.

Cluster C consists of eight members, where four came from Gojam, two from Shewa, one from Kefa and another one from Wolo. The altitude ranges from 1750-2900 m asl showing the widest range of altitude for this group. When we look at the altitude grouping of accessions two accessions belong to the first group, no accession represents the second and third altitude group, three accessions belong to the forth and three others to the fifth group.

In this analysis the highest Kruskal-Wallis value is for characters like plant height, days to 50% flowering, leaf length, days to 75% maturity and leaf width respectively. These character contribute highly to the differentiation of the accessions into the above three groups. In most of the above characters and others which have lower Kruskal-Wallis values, like dry matter and leaf stem ratio, members of cluster three show a higher result compared to the other two clusters. From Appendices 6 and 7 we can see that cluster three showed the highest mean values for most of the agronomic characters considered. Therefore accessions listed in cluster C of Table 6 can be of higher forage value than those accessions listed in cluster one and two. In relative terms, accessions in cluster two are better in their forage value than those in cluster one.

Table 5 Average linkage clustering of 50 *T. steudneri* accessions based on the 19 quantitative morphological characters.

Cluster	Accession Numbers
I	6222,8087,6261,7658,9959,8483,6253,8084,8491,9456.
II	7671,7853,9961,9956,10139,8329,10111,8338,8458,8461.
III	8357,9991,8465,10107,8450,10130,9966.
IV	7652,7659,9424.
V	7637,7677,7747,9970,7697,7779,7620,9058,7628,10125,7645,7667,9720, 8361,8357,9704,9712,8485,9700.
VI	9452.

Table 6 Average linkage clustering of 50 *T. steudneri* accessions based on eight forage agronomic characters.

Cluster	Accession Numbers
A	6222,8087,6253,6261,8483,9456,8491,9959,8084.
B	7620,7645,8361,9058,8338,9704,9720,9712,9956,10139,7658,7637,7628, 9380,10111,7637,10125,7667,8465,8329,8450,8485,10130,7659,7697, 7747,9424,8458,7677,7779,9966,9970,10107.
C	7652,9961,8357,7671,7853,9700,9991,9452.

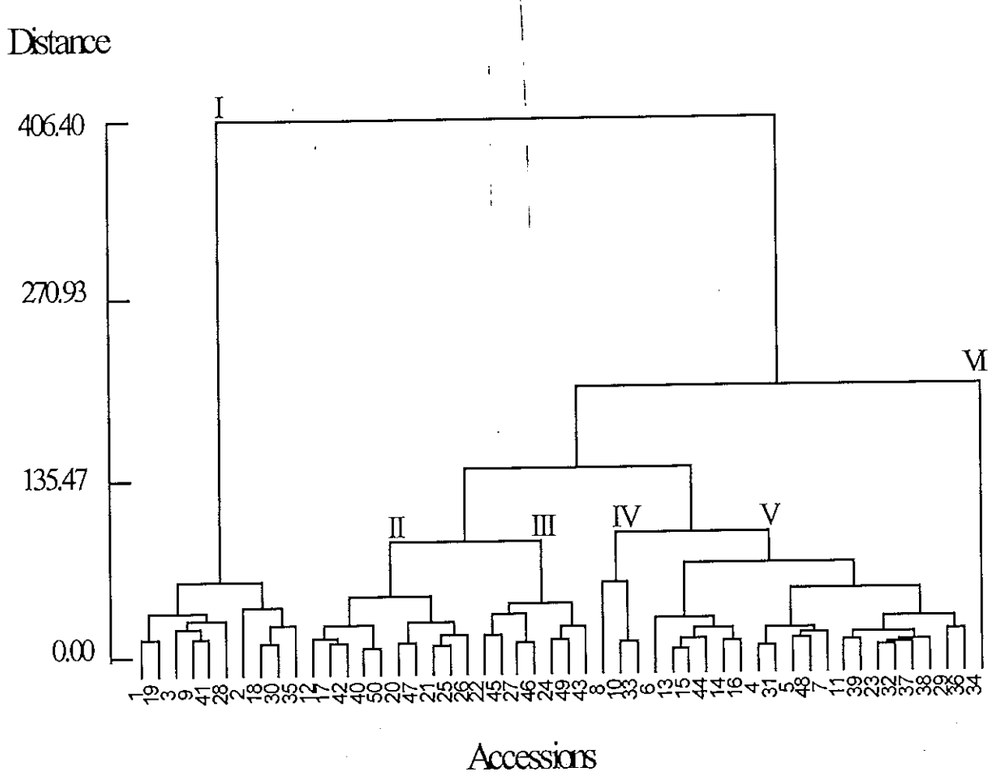


Figure 1 Dendrogram using average linkage on 50 *T. steudneri* populations based on 19 quantitative morphological characters (Refer to Table 1 for the accession numbers).

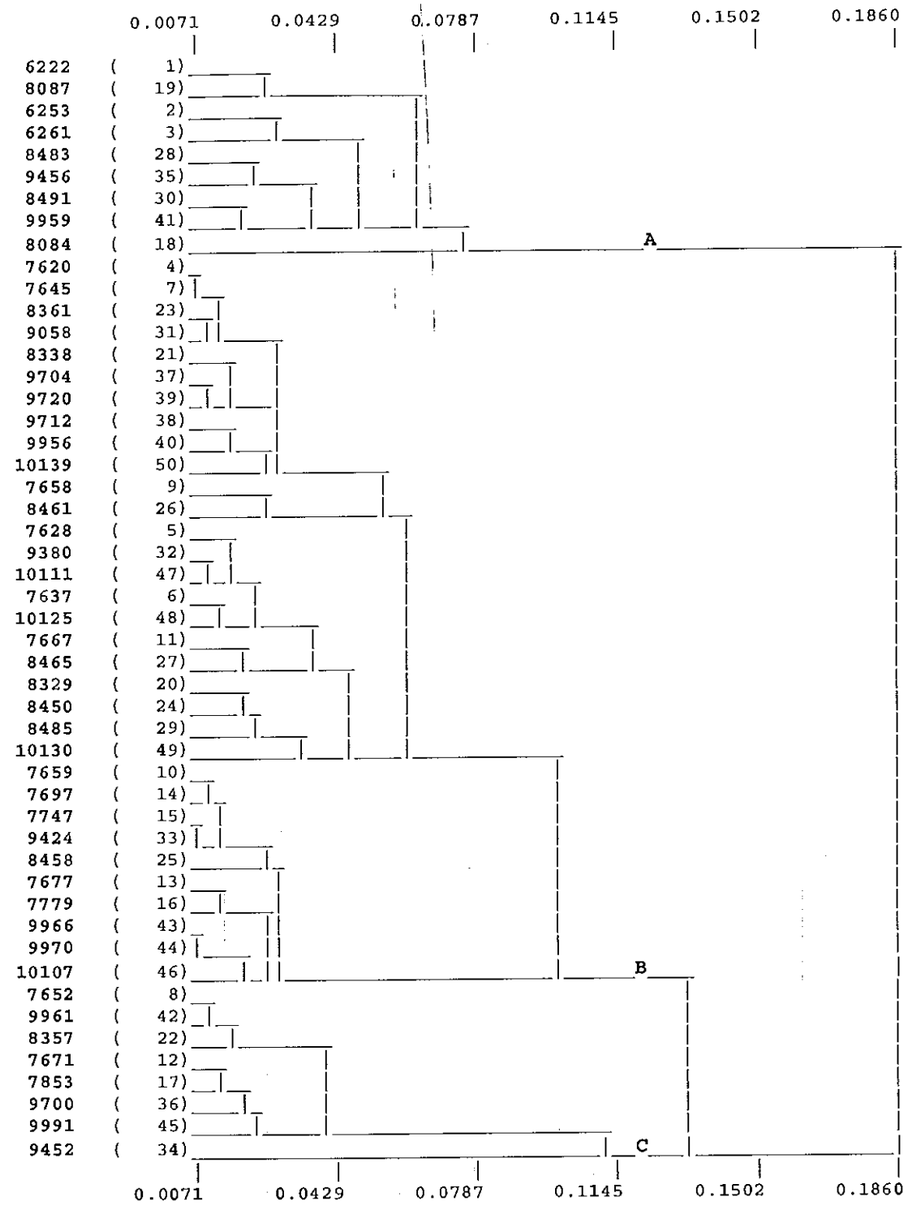


Figure 2 Dendrogram using average linkage on 50 *T. steudneri* populations based on eight forage agronomic characters.

The clustering result, using the 19 morphological characters, was further confirmed by the distance analysis (Mahalanobis Distance, D^2) among the clusters (Table 7). This analysis shows distance between clusters, where maximum distance, suggests the existence of diversity among the populations, and hence, using this information, parental lines can be selected and used for hybridization and subsequent improvement of the species.

Table 7 Mahalanobis's distance between clusters made by qualitative morphological characters.

Cluster	I	II	III	IV	V	VI
I	0.000	91.5**	134.1**	352.4**	114.8**	818.8***
II		0.000	27.4*	187.9**	23.4*	499.8***
III			0.000	205.8**	44.2**	490.2***
IV				0.000	162.2**	573.4***
V					0.000	483.9***
VI						0.000

***P<0.001, **P<0.01,* P<0.05 , ns = non significant.

Distance between cluster II and V was the minimum (D^2= 23.4), and distance between clusters I and VI was the maximum (D^2= 818.8). Minimum distance was also detected between clusters II and III (D^2= 27.4). Looking at the result of the distance between clusters, it is clearly distinct that cluster VI shows a highly significant deviation from the rest of the clusters. This result, together with the isozyme result and field trial observations, may suggest that the only member of cluster VI, accession number 9452, should be further checked whether it belongs to the species *T. steudneri* or not. It is mentioned in Sheidai *et al.* (1999) that seed protein analysis can reveal differences among populations, which can also be used to check this accession. Cluster IV shows a relatively high distance from clusters I, III and II, respectively. Parents selected from clusters with larger distance between them can result in greater variation (Haile *et al.*, 1999). Therefore crossing of

parents from cluster IV with those members of clusters I, II, III and crossing between parents from cluster I with members of clusters III, V and crossing between cluster IV and V could produce a variable recombinant.

4.1.4 Factor Analysis

According to Seiler and Stafford (1985), factor analysis is a type of multivariate analysis that can be used to reduce a large number of correlated variables to a small number of main factors. This will help to explain the number and nature of causative influences and aid in selecting better genotypes.

In this analysis it was possible to extract 13 factors, but the first six factors with an eigenvalue greater than unity were considered significant for this particular study (Table 8). The first six factors explain 75% of the total variation. To visualize the overall variability among the accessions under consideration the first two factors were plotted (Figure 3).

Table 8 Principal factor matrix after verimax rotation for 19 quantitative characters of *T. steudneri,* including Eigenvalues and total variance.

Characters	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Communality
stmthk	-0.165	-0.769	-0.124	-0.114	0.219	-0.106	0.707
indlgt	0.276	-0.152	0.173	0.890	-0.094	0.074	0.936
leflgt	0.210	-0.762	0.322	0.173	-0.180	0.186	0.825
lefwdt	0.875	-0.327	0.165	0.059	0.001	0.004	0.904
stplgt	0.828	-0.354	0.143	0.128	-0.058	0.080	0.858
stpwdt	0.846	0.104	-0.186	0.110	0.124	-0.036	0.790
flwlgt	-0.025	-0.040	-0.329	0.206	0.227	-0.534	0.490
flwwdt	0.123	0.160	-0.024	-0.053	-0.070	-0.866	0.798
flwnum	0.474	0.133	0.098	0.323	0.168	-0.333	0.495
pedlgt	0.298	-0.514	0.364	0.198	-0.469	-0.081	0.752
petlgt	0.609	0.027	-0.078	-0.190	-0.384	-0.309	0.658
sedpod	0.102	-0.084	-0.033	-0.150	-0.731	0.040	0.577
hunswt	-0.162	0.041	0.030	0.187	-0.635	0.089	0.476
flow50	-0.412	0.688	-0.383	0.145	0.171	-0.277	0.917
matu75	-0.393	0.657	-0.401	0.139	0.184	-0.251	0.863

Table 8 (Continued)

Characters	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Communality
plthgt	-0.035	0.162	-0.100	0.915	-0.016	-0.149	0.896
lfstra	-0.017	-0.056	-0.434	0.141	-0.472	-0.370	0.571
plntwt	-0.111	0.145	-0.902	-0.088	-0.017	-0.056	0.858
drymat	0.115	0.205	-0.903	-0.024	-0.038	-0.100	0.882
Eigenvalues	3.40	2.76	2.62	2.05	1.79	1.63	14.25
%of total variance	18	15	14	11	9	8	75

In Table 8, the first factor is strongly associated with traits such as leaf width, stipule length, and stipule width and to a lesser extent with flower number and petiole length. Most of the characters in this factor, except flower number, shows higher loading, which is greater than 0.6. Apparently, as shown in Figure 3, the first factor made cluster I and VI to be clearly separated from the other clusters. The first factor alone explains 18% of the total variation that exists.

The second factor, which explains 15% of the total variation, was associated with stem thickness, leaf length, peduncle length, days to 50% flowering and days to 75% maturity. These characters are important both in the forage agronomic as well as adaptation aspects of the species.

The third factor has strong association with plant weight and dry matter. These characters can be taken as important forage agronomic characters, since they have a lot to contribute to the forage value of *T. steudneri*. This factor explains 14% of the total variation that exists.

The fourth factor is again strongly associated with internode length and plant height. Both

characters in this factor, which maximized the difference among clusters, are important in the forage agronomic aspect of the species. This factor alone explains 11% of the total variation.

The fifth factor was associated with seed per pod, hundred seed weight and leaf stem ratio. Leaf stem ratio shows a relatively lower loading, which is less than 0.5. The characters in this factor are important in the reproductive aspect of the plant and this factor explains 9% of the total variation.

The sixth factor also shows association with flower length and flower width, which are again important from the reproduction point of view of the plant. These characters play a roll in increasing the divergence among clusters and this factor alone explains 8% of the total variation.

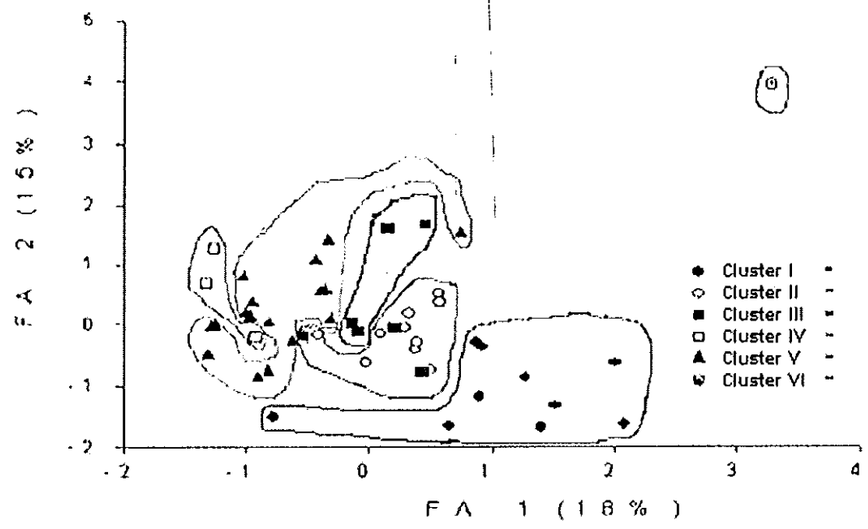


Figure 3 Ordination of the 50 *T. steudneri* populations taking the first two factors scores.

The only member of cluster VI (accession number 9452) was found to be an outlier because of its many unique characters. As shown in Figure 3, considering only the first two factors, this accession was found to have wider leaf and stipule, longer petiole and stipule, and large number of flowers. The accession was late in flowering and maturity, which also contribute for the accession to be separated from the rest.

Characters that separated Cluster I and VI from the other clusters were leaf width, stipule length and stipule width, which were greater in these two clusters. Greater values in leaf

length, peduncle length, and internode length are characters that separate Cluster I from the rest. A relatively greater value in days to 50% flowering and days to 75% maturity made cluster IV to separate from the rest, having little overlapping with cluster V. There was also marginal overlapping between cluster III and V. There was more overlapping between cluster II and III, as shown in Figure 3, which indicates the closeness of these clusters.

4.1.5 Distribution of Characters

4.1.5.1 Regional and Altitudinal Distribution of Characters

The percentage frequency of the phenotypic classes of the accessions for regional and altitudinal groups and respective chi-square values for the eight qualitative characters are given in Table 9 and 10.

Accessions from Welega and Gojam were found to show a purple flower color of type purple 82(A) (from the Royal Horticultural Society color code), at a higher frequency (Table 9). A highly significant deviation for this color in Gojam and Welega and again a highly significant deviation for purple 87(A) were observed in Gonder and Wolo accessions. Gojam is unique in having flower color type purple 87(C), relative to other regions. One accession from Gojam, accession number 9452, was found to show this type of color.

Table 9 Percentage of phenotypic classes and chi-square values for each region.

Region	flwcol				ghabit					lfmark				lwlfha			lefshp				nercol			stmhar					uplfha		
	1	2	3	X^2	1	2	3	4	X^2	1	2	3	X^2	1	2	X^2	1	2	3	X^2	1	2	X^2	1	2	3	4	X^2	1	2	X^2
Gojam	57	34	9	26.12***	27	0	28	45	30.60**	99	0	1	4.64	100	0	4.16	87	6	7	38.50***	98	2	0.46	82	18	0	0	1.98	100	0	1.42
Gonder/Wolo	36	64	0	20.29***	33	0	22	45	11.23	100	0	0	5.49	96	4	0.00	100	0	0	2.88	100	0	3.30	96	2	0	2	14.42	100	0	1.42
Kefa	43	57	0	10.13	57	8	17	18	41.79***	82	16	2	34.11***	95	5	0.26	100	0	0	2.88	92	8	7.43	67	30	0	3	18.34	100	0	1.42
Shewa	50	49	1	2.20	55	1	16	28	14.79	93	6	1	0.67	89	11	12.76*	99	0	1	1.46	94	6	2.53	69	28	1	2	15.61	92	8	22.71***
Welega	93	7	0	56.16***	17	0	66	17	63.26***	100	0	0	5.49	100	0	4.16	100	0	0	2.88	100	0	3.30	100	0	0	0	20.77	100	0	5.08

Significance level P< 0.001= ***, P< 0.01= **, P< 0.05 = *, otherwise ns (not significant)

Table 10 Percentage of phenotypic classes and chi-square values for each altitude groups.

ALT. group	flwcol				ghabit					lfmark				lwlfha			lefshp				nercol			stmhar					uplfha		
	1	2	3	X^2	1	2	3	4	X^2	1	2	3	X^2	1	2	X^2	1	2	3	X^2	1	2	X^2	1	2	3	4	X^2	1	2	X^2
≤1850	44	44	12	18.82*	70	4	21	5	45.39***	90	8	2	5.50	100	0	3.30	86	12	2	39.69***	96	4	0.01	83	17	0	0	3.68	94	6	0
1851-2050	51	45	4	0.02	33	0	13	54	18.96	99	0	1	4.42	100	0	3.30	93	0	7	6.23	100	0	4.38	84	13	0	3	5.89	96	4	0.71
2051-2250	41	58	1	8.27	66	0	7	27	21.49*	90	10	0	9.07	86	14	37.65***	99	0	1	4.34	89	11	11.49*	51	43	3	3	41.07***	83	17	21.45***
2251-2450	55	43	2	1.13	34	0	26	40	6.47	96	3	1	0.40	98	2	0.47	97	0	3	2.53	100	0	4.38	85	14	0	1	4.35	98	2	2.84
≥2451	67	33	0	11.41	19	1	37	42	27.19**	100	0	0	5.26	100	0	3.30	96	0	4	2.54	94	6	0.81	79	20	0	1	1.01	99	1	4.43

Significance level P< 0.001= ***, P< 0.01= **, P< 0.05 = *, otherwise ns (not significant)

- Description of the scores:

flowcol- Purple 82(A) = **1**, Purple 87(A) = **2**, Purple 87(C) = **3**
ghabit- Creeping-ascending = **1**, Semi-erect = **2**, More semi-erect and creeping-ascending = **3**, More creeping-ascending and semi-erect = **4**
lfmark- No leaflet mark = **1**, Mark at leaflet tip = **2**, Mark at leaflet base = **3**
lwlfha \ uplfha- Smooth = **1**, Sparse soft hairs = **2**
stmhar- Smooth = **1**, Sparse soft hairs = **2**, Medium dense soft hairs = **3**, Dense soft hairs = **4**
lefshp- Linear = **1**, Oblong = **2**, Linear and pointed = **3**
nercol- Lighter = **1**, Darker = **2**

Concerning growth habit (form), accessions from Welega were more semi-erect but still creeping-ascending type. Creeping-ascending types were found in high frequency in Kefa and Shewa. Gojam, Gonder and Wolo regions were found to have more of the creeping-ascending but semi-erect types. Kefa is unique in having a strictly semi-erect type of accessions, although a highly significant deviation for creeping-ascending types is found in Kefa. A highly significant deviation was also observed for semi-erect but creeping-ascending types in Welega. On the other hand, significant deviation is detected for creeping-ascending but semi-erect type in Gojam.

A brown mark at the tip of the leaflet is a unique feature for some accessions from Kefa and Shewa. Some accessions from the afore-mentioned regions and Gojam also show a brown leaf mark at the base of their leaflets. Accessions from region Kefa showed a highly significant deviation for brown mark at the tip of leaflets.

Sparse soft hair was a character observed in some accessions of Gonder, Wolo, Kefa and Shewa, for lower leaflet surface hairiness. In most cases the lower leaflet surface was found to be smooth. A slightly significant deviation from the expected distribution was detected for the sparse soft hair character in Shewa.

When it comes to leaflet shape, most of the regions show a linear type but accessions from Gojam were found to have an oblong type as well as linear with pointed types. This is indicated in the highly significant deviation from the expected distribution for oblong and linear with pointed apex characters in accessions from Gojam.

Light leaflet nerve color is a common feature in most of the regions. Even though this is the case, some accessions from Gojam, Kefa and Shewa show a darker leaflet nerve color, but with no significant deviation from the expected.

Similarly, stem hairiness did not show significant deviation from the expected distribution in any of the regions. Sparse soft hair is found in a relatively higher frequency in accessions from Kefa and Shewa. Upper leaflet hairiness showed a highly significant deviation for sparse soft hair in accessions from Shewa. The upper leaflet surface was smooth in all the accessions from other regions.

Flower color shows that accessions in the lower altitude have a higher frequency of flower color of purple 87(C) than the other altitude classes (Table 10). This makes the class to have slightly significant deviation from the expected for this color. For the higher altitude areas purple 82(A) is found in a higher frequency, but without any accession for the purple 87(C).

The Creeping-ascending type of growth is most common in the lower altitude areas (≤1850 m asl) and medium altitude areas (2051- 2250 m asl). Accessions from those areas have highly significant deviation and slightly significant deviation from the expected distribution. For the higher altitude areas (≥2451 m asl) accessions with the creeping-ascending but semi-erect type of growth habit (form) were in relatively higher frequencies. This altitude class significantly deviates from the expected distribution for creeping-ascending but semi-erect type of growth.

4.1.5.2 Diversity

Estimates of diversity (using Shannon-Weaver diversity index, H') for individual characters, regions and altitude classes are shown below. Regarding the qualitative traits, some of the characters show polymorphism but characters like calyx shape were found to be a taxonomic character, which was quite similar in all the individuals studied.

The highest mean diversity index pooled over characters was for Shewa (H'=0.43 ± 0.07), followed by Kefa (H'=0.41 ± 0.09) (Table 11). The highest mean diversity index pooled over altitude ranges was for class 2051-2250 m asl (Table 12). The over all diversity index was calculated to be 0.40± 0.07.

Table 11 Estimate of mean diversity (H') and standard errors of the accessions for characters and regions.

Region	flwcol	ghabit	lfmark	lwlfha	lefshp	nercol	stmhar	uplfha	H' ±SE
Gojam	0.82	0.77	0.06	0.00	0.42	0.14	0.34	0.00	0.32±0.16
Gonder/Wolo	0.41	0.78	0.001	0.26	0.001	0.00	0.27	0.29	0.21±0.13
Kefa	0.62	0.82	0.48	0.29	0.001	0.41	0.62	0.00	0.41±0.09
Shewa	0.66	0.74	0.23	0.49	0.07	0.33	0.53	0.37	0.43±0.07
Welega	0.22	0.63	0.001	0.00	0.001	0.00	0.001	0.00	0.11±0.12
Total	0.74	0.81	0.20	0.32	0.21	0.25	0.45	0.22	0.40±0.07

Table 12 Estimate of mean diversity (H') and standard errors of the accessions for characters and altitude classes.

Altitude	flwcol	ghabit	lfmark	lwlfha	lefshp	nercol	stmhar	uplfha	H' ±SE
≤1850	0.89	0.62	0.34	0.00	0.42	0.25	0.34	0.32	0.40±0.12
1851-2050	0.76	0.70	0.07	0.00	0.22	0.00	0.37	0.24	0.30±0.14
2051-2250	0.66	0.58	0.30	0.58	0.07	0.51	0.66	0.67	0.50±0.03
2251-2450	0.71	0.78	0.18	0.15	0.11	0.00	0.31	0.15	0.30±0.15
≥2451	0.58	0.82	0.00	0.00	0.15	0.31	0.39	0.09	0.30±0.12
Total	0.74	0.81	0.20	0.32	0.21	0.25	0.45	0.22	0.40±0.07

4.1.6 Estimate of components of variance, heritability (broad sense) and genetic advance

Table 13 shows summary statistics on the phenotypic and genotypic coefficients of variance (PCV and GCV), estimate of the component of variance, broad sense heritability, and genetic advance as percent of the mean. Looking at the results from the Table, the phenotypic coefficient of variation is higher than the genotypic coefficient of variation, especially in some forage agronomic characters like dry matter, plant weight and plant height. This shows that genotypic factors play an important role in controlling the existing variation.

The forage agronomic characters show wide differences between their values of PCV and GCV, which indicate the complexity of the characters and the importance of other factors, for instance environment, on expression of those traits, in addition to the genetic factors.

The higher heritability value for most of the morphological quantitative characters shows the lesser influence of environment in most of these characters. The relatively lesser heritability value for stem thickness and flower width shows the influence of environment on expression of these characters.

4.2 ISOZYME STUDY

4.2.1 Population variability

Table 14 shows the genetic variability of 17 loci in the accessions selected for the study. Mean number of alleles per locus was found to be 1.5 for three accessions (9452, 7652 and 8084) and 1.6 for six accessions (6222, 7677, 7747, 8461, 9991 and 10111). The percentage of polymorphic loci range from 64.7 to 47.1. Accessions 9452 and 7652 had the highest percentage of polymorphic loci. On the other hand, accessions 6222, 7747,9991 and 7677 have the lowest percentage. In all the cases a locus is said polymorphic if more than one allele was detected. As to this criterion three loci (ESA-6, PRX-2 and ESB-5) were represented by one allele each and, therefore cannot be considered as polymorphic. Except in these loci, polymorphism was detected in all loci. Accessions collected from Gojam region show the highest polymorphism (both accessions, 9452 and 7652 are from Gojam). Mean heterozygosity for the populations studied range from 0.199 to 0.337. The highest range goes to accession 8084 and the lowest to accession 7747. Existing variation between and within accessions using the four enzyme systems is illustrated in Figures 4 to 7. Appendix 8 indicates the number and type of alleles in each locus for each accession included in the isozyme study.

Table 14 Genetic variability at 17 loci in nine populations.

Accession no.	Mean no. of alleles per locus	Percentage of loci polymorphic	Expected Aveerage of population heterozygosity
1. 9452	1.6	64.7	.244
2. 6222	1.5	47.1	.224
3. 7747	1.5	47.1	.199
4. 9991	1.5	47.1	.238
5. 10111	1.5	52.9	.280
6. 8461	1.5	52.9	.277
7. 7677	1.5	47.1	.218
8. 7652	1.6	64.7	.304
9. 8084	1.6	58.8	.337

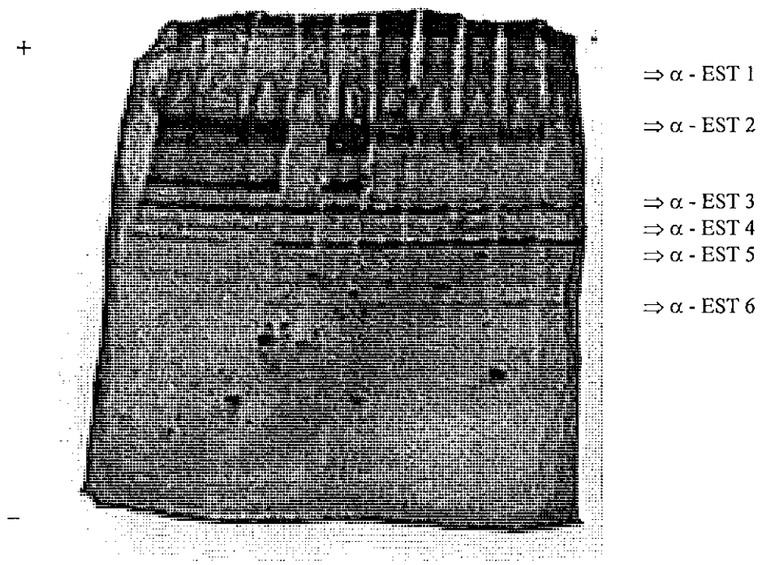


Figure 4 Gel for α-Esterase enzyme (Acc. No. 9452 and 6222, each with five plants from left to right).

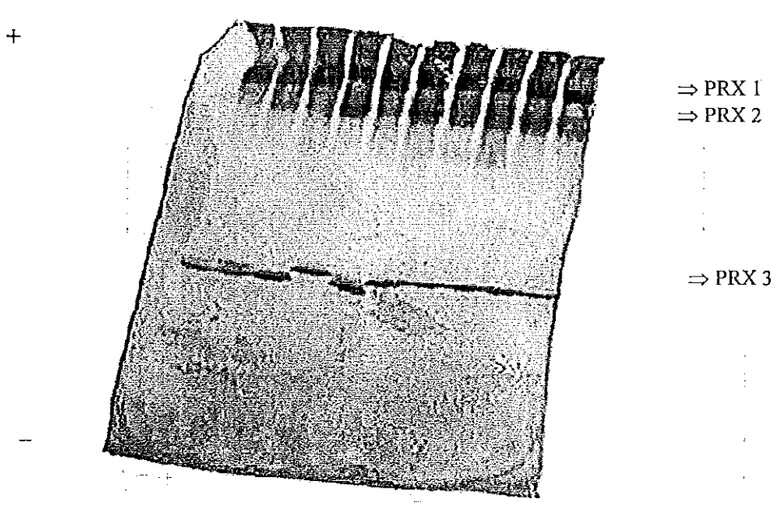


Figure 5 Gel for Peroxidase enzyme (Acc. No. 9452 and 6222, each with five plants from left to right).

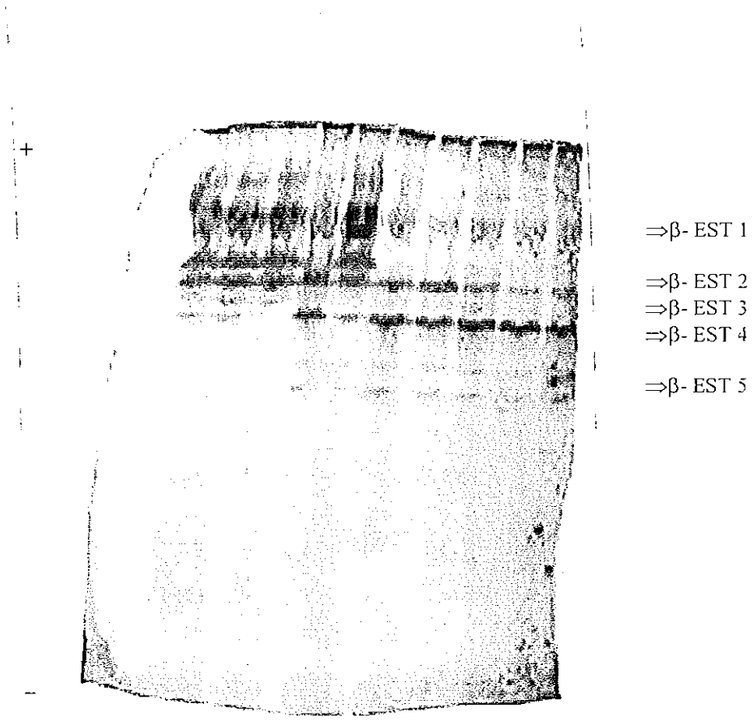


Figure 6 Gel for β-Esterase enzyme (Acc. No. 9452 and 6222, each with five plants from left to right).

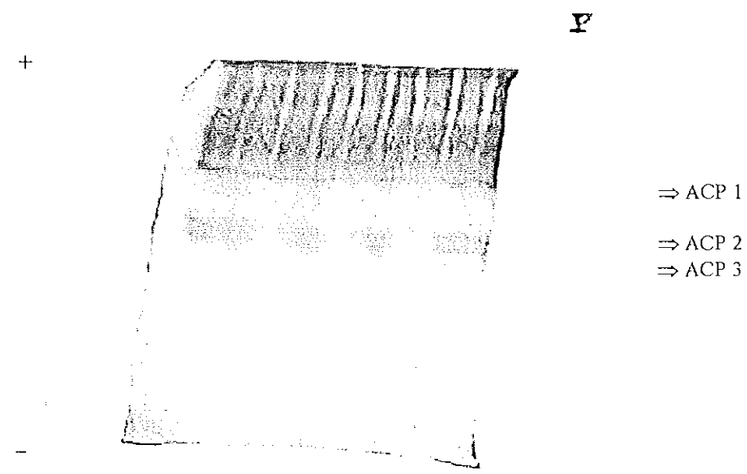


Figure 7 Gel for acid phosphatase (Acc. No. 7652 for the first four and Acc. No. 8084 for the next five consecutive plants, from left to right).

accessions came from a same altitude class (≤ 1850 m asl), they were physically located far away from each other.

Table 16 Matrix of genetic distance coefficients.

Below diagonal: Nei (1978) unbiased minimum distance.

Population		1	2	3	4	5	6	7	8	9
1	9452	*****								
2	6222	.152	*****							
3	7747	.076	.108	*****						
4	9991	.113	.061	.007	*****					
5	10111	.092	.068	.025	.000	*****				
6	8461	.090	.073	.021	.000	.000	*****			
7	7677	.079	.099	.000	.002	.003	.001	*****		
8	7652	.035	.130	.051	.036	.015	.014	.044	*****	
9	8084	.141	.015	.119	.084	.055	.061	.091	.119	*****

Accessions were clustered using unbiased minimum distance by the unweighted pair group method (Fig. 8). The clustering result was almost similar with the clustering result obtained from cluster analysis of the morphological characters. Four major clusters were identified by cluster analysis of the nine accessions in the isozyme analysis, using the Biosys software (Swofford, and Selander, 1981). **Cluster W** and **Y** have a single member each, **Cluster X** with five members and **Cluster Z** with two members.

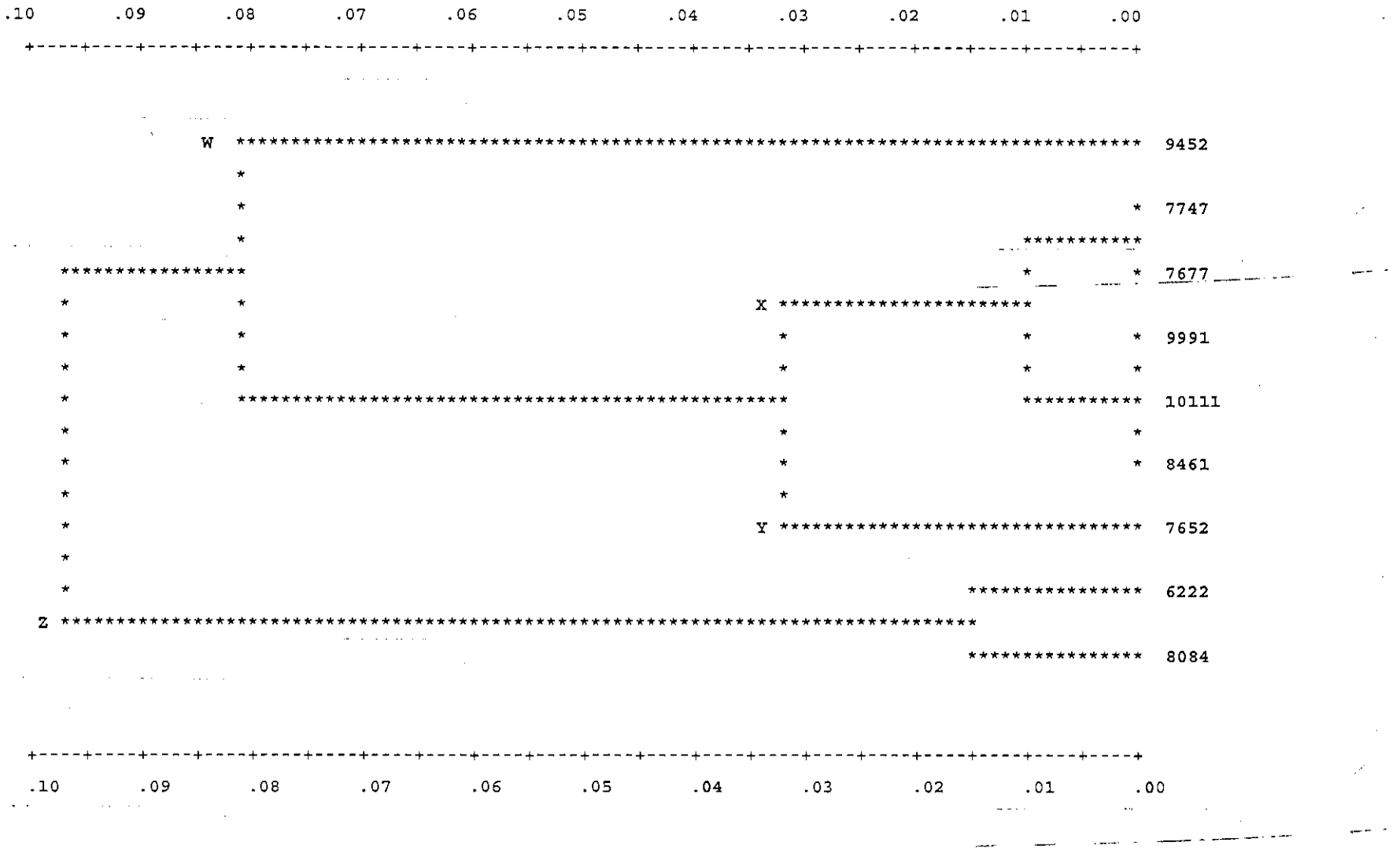


Figure 8 Dendrogram of the clustering of nine *T. steudneri* populations based on the isozyme results.

5. DISCUSSION

5.1 REPRESENTATIVENESS OF ACCESSIONS

It was mentioned by Thulin (1983) and (Thulin and Hunde, 1989) in the Flora of Ethiopia that *T. steudneri* is one of the widely distributed *Trifolium* species in the highlands of Ethiopia. These areas include Shewa upland, Tigray upland, Gonder upland, Arsi and Kefa. Based on this, the 50 accessions used in this study may not be large enough to capture all the variation that exists. But, as shown in the GIS output (Map 1), the selection of the 50 accessions was made to be as representative as possible from the existing 85 collections in the genebank. Map 2 is a GIS output, which indicates that most of the accessions were collected from Vertisol areas.

5.2 MORPHOLOGICAL CHARACTERIZATION

The mean value is widely known to be used in determining variation in agronomic characters found within and between populations (Jaradat, 1991; Endashaw Bekele, 1996; Belay, 1997). ANOVA showed a significant difference between accessions using the mean values for most of the quantitative morphological characters considered. From the PATN analysis, by looking at the Kruskal-Wallis value for the characters, it was possible to find out those characters, which contributed most for the variability.

Correlation analysis in the study showed positive and negative correlations between characters. For instance higher positive correlation between leaf width and stipule length shows that increase in leaf width appear together with increase in stipule length. On the other hand leaf size is negatively correlated with the lateness of accessions. This means that the accessions with small leaves tend to be late in flowering and maturity or vise versa.

Those characters, which were highly correlated like, plant height and internode length, and days to 50% flowering and days to 75% maturity, were among those, which contributed highly for the variation. Therefore, these characters were important in clustering the 50 accessions into six clusters.

Cluster I, except for accession no.7658 and 9456 (which are from Gojam), all accessions are from Shewa, and were similar phenotypes from a similar environment. The cluster contains accession no. 6261, which is from a low rainfall area of Shewa, showing the possibility of using this accession in semi-arid environments. The range of altitude for this cluster is relatively wider than cluster III that shows a wide altitude adaptation. The dominant soil type from which members were collected is a Vertisol type with clay texture, showing the adaptation of members of this cluster to waterlogging. Collections were made from acid soils with the exception of two Shewa accessions, which were collected from sites with neutral soil pH (Acc. No. 8491 and 8087). Most members of this cluster show an adaptation to a seasonal waterlogged area. Protected areas such as borderline between two crops fields were also areas where members of this cluster are found, showing their members may tolerate grazing. Accession number 7658 was collected along a water ditch that can show the possibility of seed dispersal mechanism by water.

Cluster II accessions, which were from five of the regions (districts) included in the study except Gonder, showed wider adaptation. Similar phenotypes can be found in different environments due to the seed dispersal mechanisms of the species. Apart from seed dispersal by water, it has been observed from experiments that pods and the mature sharp calyx tend to have a highly coarse surface, which makes it easier for the seed to be attached

to animals and transported to other places. The growth habit (form) of most of the accessions being decumbent (creeping-ascending branches with a meristem at the tip), allows them to branch far in between cultivated crops and be harvested together with that crop. These characters promote seed dispersal for the species to be found in different environments of the highlands. In this cluster we find accessions like 7853 and 7671, which are from the highest altitude, showing their adaptation to higher rainfall areas. The lowest altitude for this cluster is for accession 8458. The range of altitude between accession from the highest and the lowest altitude is higher in this cluster, which is about 1080m. Accessions in this group were collected from different soil types, which have a clay texture. Most of the accessions in this cluster are from acidic soils and were found in croplands like Noog, Sorghum and Teff fields. They can also grow on fallows. This shows that grazing areas are suitable sites for *Trifolium*.

Cluster III contains accessions from Shewa, Kefa and Welega, and again show a relatively wide adaptation. Accessions 8450 and 9991 are from the lowest altitude areas of all the accessions in the study. Most of the accessions are from a similar type of soil, which is relatively acidic. The accession from Kefa-Jimma (accession number 9991) is from an area with a high temperature of about 19.9^{0}C, a lower altitude of 1750m asl and a high rainfall of about 1500mm. Therefore this accession is adapted to warm humid areas.

Cluster IV contains three members, which are all from Gojam, with similar phenotypes being found in similar environments. They are from higher altitude areas (2500-2520m asl), with a minimum altitude range between them. The accessions are from acidic Vertisol

clays. Just like other groups, this group is also adapted to areas of high moisture and protected environments.

Cluster V includes accessions with the most common phenotype collected, with the highest number of members, which are from Gojam, Gonder and Shewa. Accessions from this cluster came from a wide range of altitude. Most members in this cluster were collected from Vertisols of various textures. Accessions such as 7697 and 9970 were collected growing in water ditches, indicating the most likely seed dispersal mechanism as water. The seed yield was found to be very low for accession number 7697, due to a smaller number of flowers.

Cluster VI contains accession number 9452 as the only member. This was found to be an uncommon phenotype for *T. steudneri*. During the field trial this accession showed a very good plant biomass and hence is a potential forage legume. It showed unique characters, which made it an outlier in the statistical analysis. The accession was exceptionally late to germinate, flower and mature. The oblong leaflet shape and vigorous stands of this accession were very distinct in the three replications of the trial. The toothed and clasping type stipule of this accession was the other unique features, which was not found in any of the accessions in the trial. Higher Mahalanobis distance value of this accession also indicates the distant relatedness of this accession from the rest.

Factor analysis showed that by plotting the first and the second factors, it was possible to show that accession number 9452 to be an outlier because of its lateness in flowering and maturity as well as by having wider leaflets and stipules. Members of cluster I and the outlier accession were found on the same axis for the first factor, since they also have wider

leaflets and stipules. But for the second factor, unlike the outlier accession, members of cluster I were found on the negative side of the axis, since they were early in flowering and maturity.

Flower color was unique for accession number 9452, which was purple 87(C) as indicated by the Royal Horticultural Society color code. It is this accession, which made Gojam region to be significantly different in the distribution of flower color.

5.3 AGRONOMIC CHARACTERIZATION

Clustering using the eight agronomic characters resulted in three clusters, which can be used as an indicator of the potential forage value of the accessions. **Cluster A** consisted of nine accessions with mean value for the measured agronomic characters less than the total mean. This would indicate their low forage productivity potential at Debre Zeit. Except accession number 9456, all came from Shewa. **Cluster B** is the largest, with 33 members, having most of the mean values for the measured characters close to the total mean value. This indicates the cluster to have members with intermediate forage productivity potential for Debre Zeit. **Cluster C**, which includes accession number 9452, has eight members whose mean values are greater than the total mean for most of the agronomic characters. This was also observed during the field trial from the vigorous stands of the accessions in this cluster. Accessions in this cluster are well adapted to Debre Zeit and show high agronomic value for that area.

Since hairiness affects the palatability of forage plants, the smooth texture of leaf and stem of *T. steudneri* in most of the accessions studied is a desirable character of a forage plant.

Cluster X of the isozyme clustering had two sub-clusters. The first sub-cluster contained accession 7747 from Gonder and 7677 from Gojam. These accessions belong to cluster V of the morphological clustering. The second sub-cluster contained three accessions 10111 from Welega, 8461 and 9991 both from Kefa. Therefore this sub-cluster contains members of cluster II (acc. no. 10111 and 8461) and III (acc. no. 9991) of the morphological clustering together. These two clusters (II and III) were close in the morphological clustering; hence most of the results of the isozyme clustering support the morphological clustering. This indicates that the enzymes tested were responsible for the expression of most of the morphological quantitative characters in the study.

accessions were found to perform best on light clay-loam soil of Debre Zeit. This area is included in the eco-climatic zone of sub-tropical grassland. Most of these accessions came from a high altitude area, which may indicate that high forage value accessions came from high altitude areas of the highland.

Leaflet and stem hairiness affect the palatability of forage plants. Most of the accessions included in the study did not show leaflet or stem hairiness. But some accessions from Shewa, such as accession number 6253, 8084,8087,8483 and 8491, were found to show leaflet hairiness. Besides, these accessions are members of cluster A in the agronomic characterization, which has low productivity. This also shows that members of cluster I have low forage value.

To exploit the variation that exists between accessions for breeding purposes, it was important to consider the existing distance between clusters. From the Mahalanobis's distance, for instance, it is possible to cross members from cluster I with cluster V or cluster III with cluster V to produce a recombinant with variable characteristics.

The accessions used in the study were representative of only small area of the wide distribution of *T. steudneri* as indicated in the Flora of Ethiopia (Thulin and Hunde, 1989). The GIS analysis showed that most of the collections were made close to main roads (Map 3). This indicates that there are still ecogeographic gaps, where collections need to be made from. In this study, the 50 accessions were randomly selected to be as representative as possible from the 85 indigenous *T. steudneri* accessions available from the ILRI forage gene bank. Even though the overall diversity index of the study was small (H'= 0.40 ±

0.07), the value might be increased by having more collections from areas, which were not covered by previous collections.

7. REFERENCES

Abebe Demissie (1996). Morphological and molecular markers diversity in Ethiopian landrace Barleys: Implication to *in situ* and *ex situ* conservation of landrace materials. *Doctor Scientiariom Thesis*. Agricultural University of Norway.

Abebe Demissie and Bjørnstad, Å. (1996). Phenotype diversity of Ethiopian barely in relation to geographical regions, altitude range and agro-ecological zone: as an aid to germplasm conservation strategy. *Hereditas* **124**: 17-29.

Akundabweni, L. (1984a). Forage potential of some annual native *Trifolium* species in the Ethiopian highlands. *Ph.D. Thesis*. South Dakota State University, U.S.A. Pp. 10-64.

Akundabweni, L. (1984b). *Native clovers of the Ethiopian highlands: the backward champions*. ILCA Newsletter **3**, No.1.

Ayoub, A.T. (1986). The potential contribution of some forage crops to the nitrogen budget and animal feed in the Sudan Gezira farming system. **In**: Haque, I., Jutzi, S. and Neate, P.J.H. (eds.), *Potentials of forage legumes in farming systems of sub-Saharan Africa*. Proceedings of a workshop held at ILCA, Addis Ababa, Ethiopia, 16-19 September 1985. International Livestock Center for Africa, Addis Ababa, Pp. 58-68.

Bailey, T.C. (1994). A review of statistical spatial analysis in geographic information system. **In**: Fortheringham, S. and Rogerson, P. (eds.), *Spatial Analysis and GIS*. Taylor and Francis, London.

Belay, G. (1997). Genetic variation , breeding potential and cytogenetic profile of Ethiopian tetraploid wheat (*Triticum turgidium* L.) landrace. *Doctoral thesis*, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Belbin, L. (1988). *PATN, Pattern Analysis Package*. Division of Wildlife and Rangelands Research. CSIRO.

Bogdan, A.V. (1977). *Tropical pasture and fodder plants*: Longman Group Press, New York. Pp.414.

Britten, E. J. (1962). Evaluation for pasture purposes of some African clovers, a plant introduction program. *J. Range Mgnt.* **15** (6): 329-333.

Brown, A.H.D. (1995). The core collection at the crossroads. **In**: Hodgkin, T., Brown, A.H.D. Van Hintum, Th.J.L. and Morales, E.A.V. (eds.), *Core collections of plant genetic resources*. International Plant Genetic Resource Institute (IPGRI). A Wiley-Sayce Publication.

Chamberlain, J. R. (1998). *Systematics and Population Genetics Laboratory Protocols for Isozyme Analysis*. London.

Eeghen, M. Van (1984). *Trifolium tembense: an explanatory study on its value as a fodder crop and short literature review on the indigenous clovers of East Africa*. Study report for ILCA, highland program, Addis Ababa.

El-Kholy, M. A. K. (1990). *Cytology of some Trifolium* species. *Ph.D. Thesis*. Al-Azhar University, Egypt.

Endashaw Bekele (1996). Morphological analysis of *Eragrostis teff*: Detection for regional pattern of variation. *SINET*: Ethiopian J. Sci. 19(1): 117-140.

Evans, T.R. (1967). Primary evaluation of grasses and legumes for the Northern Wallum of South-east Queensland. *Tropical grasslands* **1**:143-152.

Farnilton, A.S. (1990). Animal disorders arising from consumption of pasture. **In**: Langer, R.H.M. (ed.), *Pastures, their ecology and management*. Oxford University Press, Auckland.

Gryseels, G. and Anderson, F. M. (1983). Research on farm and livestock productivity in the central Ethiopian highlands: Initial results, 1977- 1980. International Livestock Center for Africa, Addis Ababa. ILCA Research reports No.4. P.52.

Guarino, L., Jarvis, A., Hijmans, R.J. and Maxted, N. (2002). Geographic Information Systems and the conservation and use of plant genetic resources. **In**: Engles, J.M.M., Ramanatha Rao, V., Brown, A.H.D. and Jackson, M.T. (eds.), *Managing Plant Genetic Diversity*. IPGRI. CABI publishing. Wallingford, New York.

Hartl, D.L. (1980). *Principles of population genetics*. Sinauer Associates, Inc. Sunderland, Massachusetts. Pp.130-139.

Heichel, G.H., Vance, C.P. and Barnes, D.K. (1983). Symbiotic nitrogen fixation of alfalfa, Birdsfoot trefoil and red clover. **In**: J.A. Smith and V.W. Hays (eds.), *Proceedings of the XIV International Grassland Congress*, held at Lexington, Kentucky, USA. June 15-24, 1981. Westview Press, Boulder Colorado, pp.336-338.

Hijmans, R.J., Schreuder, M., Cruz, J. De La and Guarino, L. (1999). Using GIS to check co-ordinates of genebank accessions. *Genetic Resource and Crop Evolution* 46: 291-296.

Hijmans, R.J., Guarino, L., Cruz, M. and Rojas, E. (2001). Computer tools for Spatial analysis of plant genetic resources data: 1. DIVA-GIS. *Plant Genetic Resource Newsletter*. No. 127:15-19.

Hijmans, R. J., Guarino, L., Bussink, C. and Rojas, E. (2002). DIVA-GIS. Version 2, Manual. *A geographic information system for the analysis of biodiversity data.* International Potato Center (CIP). Lima, Peru.

Humphreys, L.R. (1981). *Environmental adaptations of plants.* McMillan Publishers, New York.

Hussain, A., Bushuk, W., Ramirez, H. and Roca, W. (1988). *A practical guide for electrophoretic analysis of isozymes and proteins in Cassava, field Beans and Forage legumes*. Food and Science Departement, University of Manitoba, Winnipeg, Manitoba, Canada and Biotechnology Research Unit, CIAT, Cali, Colombia.

IBPGR, 1987. IBPGR Training Courses: Lecture Series. 1. *Collection, Characterization and Utilization of Genetic Resources of Temperate forage Grass and Clover*. IBPGR, Rome.

IBPGR, 1992. *Descriptor for White clover* (*Trifolium repens* L.). International Board for Plant Genetic Resources. Rome, Italy.

Isermann, K.(1983). Quantitative and Qualitative aspects of the energy requirement for the nutrition of crop plants with technically, atmospherically and biologically fixed nitrogen. *Plant Res. and Development* **17:** 7-22.

Jahnke, H.A. 1982. *Livestock production systems and livestock development in tropical Africa.* Kieler Wissenschaftsverlag Vauk, West Germany.

Jaradat, A. (1991). Phenotypic divergence for morphological and yield related traits among landrace genotypes of durum wheat from Jordan. *Euphytica* **52**: 155-164.

Kahurananga, J. (1981). *A proposal on the exploration of indigenous forage legumes.* ILCA research note No. 1, Highland program, Addis Ababa, Ethiopia.

Kahurananga, J. (1982). ILCA's forage legume work in the Ethiopian highlands. ILCA Newsletter **1**, No.4.

Kahurananga, J. (1991). Inter-cropping Ethiopian *Trifolium* species with Wheat. *Experimental Agriculture* (UK). **27**(4): 385-390.

Kamara, C.S. and Haque, I. (1988). *Characteristics of upland soils at ILCA research sites in Ethiopia* (No. B6). Soil and Plant Nutrition Section. International Livestock Research Center for Africa, Ethiopia.

Mannetje, L. and Pritchard, A. J. (1974). The effect of day length and temperature on introduced legumes and grasses for the tropics and sub-tropics of costal Australia: I. Dry matter production, tillering and leaf area. *Aust. J. of Expl. Agric. and Anim. Husb.*

Murfet, I. C. (1977). Environmental interaction and genetics of flowering. *Ann. Rev. Plant Physiol.* **28**: 253-278.

MINITAB. (1998). *MINITAB. User's guide, Release 12.22.* MINITAB Inc.

MSTATC. (1991). *MSTATC User's guide Version2.1.* Michigan University.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583-590.

Norris, D.O., and Mannetje, L. (1964). The symbiotic specialization of African *Trifolium* species in relation to their taxonomy and their agronomic use. *E. Afric. Agric. For. J.* **62**(3): 112-120.

Pritchard, A.J. (1972). Number and morphology of chromosomes in African species in the genus *Trifolium*. *Aust. J. Agric. Res.* **13**: 1023-1029.

Pritchard, A.J. and Mannetje, L. (1967). The breeding system and some interspecific relations of a number of African *Trifolium* species. *Euphytica* 16(3): 324-329.

Sanchez, P.E. (1976). *Properties and management of soil in the tropics*. John Wiley and sons, London.

SAS. (1999). *SAS users guide*: Statistics Version 8. SAS Institute. Cary, NC.

Scandalios. J.G. (1974). Isozymes in development and differentiation. *Ann. Rev. Plant Physiol.* **25**: 225-258.

Seiler, G. J. and Stafford, R. E. (1985). Factor analysis of components in Guar. *Crop Science*. **25**: 905-908.

Sheidai, M., Hamta, A., Jaffari, A. and Noori-Daloii, M.R. (1999). Morphometric and seed protein Studies of *Trifolium* species and cultivars in Iran. Plant Genetic Resources Newsletter. No. **120**: 52-54.

Simpson, M.J.S. and Withers, L.A. (1986). *Documentation of genetic resources. Characterization using isozyme electrophoresis: A guide to the literature.* IBPGR. Pp.102.

SPSS (1999). *SPSS for Windows Release 9.1*. SPSS. Inc.

Swofford, D. L. and Selander, R. B. (1981). *BIOSYS-1 Release-1. A Computer Program for the Analysis of Allelic Variation in Genetics*. User's Manual. University of Illinois at Urbane Champaign, Urbane, U.S.A.

Tesfayesus Seiro (1992). *Climatic records for ILCA research sites*. ILCA headquarters, Addis Ababa, Debre Zeit ILCA station, Debre Berhan ILCA station, Soddo (WADU). International Livestock Center for Africa, Ethiopia.

and Williams, J.T. (eds.), *Crop genetic resources: Conservation and evaluation*. IBPGR. George Allen and Unwin, London, Boston, Sydney.

Wouw, M. Van De ; Hanson, J. and Nokoe S. (1999). Observation strategies for morphological characterization of forages. *Genetic resource and crop evolution*. **46**: 63-71.

Young, A. (1980). *Tropical soil and Soil Survey*. Cambridge University Press, Cambridge, England. Pp.180-192.

Zohary, M. (1972). Origin and evolution in the genus *Trifolium*. *Bot. Notiser*.**125**: 501-511.

8. APPENDICES

Appendix 1 List of accessions used in the study (From the passport data of *T. steudneri* ILRI Forage Genetic Resource Project).

No.	Acc. No.	Old Region	Old District (Awraja)	New Region	Altitude
1	6222	Shewa	Addis Ababa	14	1800 *
2	6253	Shewa	Menagesha	4	2380
3	6261	Shewa	Addis Ababa	14	1800 *
4	7620	Shewa	Selale	4	2550
5	7628	Gojam	Debre Markos	3	2420
6	7637	Gojam	Debre Markos	3	2420
7	7645	Gojam	Bichena	3	2520
8	7652	Gojam	Mota	3	2520
9	7658	Gojam	Mota	3	2450
10	7659	Gojam	Bichena	3	2510
11	7667	Gojam	Bichena	3	2500
12	7671	Gojam	Bichena	3	2610
13	7677	Gojam	Debre Markos	3	2400
14	7697	Gojam	Debre Markos	3	2350
15	7747	Gonder	Gonder	3	2140
16	7779	Gonder	Debre Tabor	3	2460
17	7853	Wolo	Ambassel	3	2900
18	8084	Shewa	Yerer & Kereyu	4	1850
19	8087	Shewa	Yerer & Kereyu	4	1780
20	8329	Shewa	Jibat & Mecha	4	2240
21	8338	Shewa	Jibat & Mecha	4	2210
22	8357	Shewa	Jibat & Mecha	4	2370
23	8361	Shewa	Jibat & Mecha	4	2260
24	8450	Kefa	Jimma	4	1750
25	8458	Kefa	Jimma	4	1820
26	8461	Kefa	Jimma	4	1950
27	8465	Shewa	Chebo & Gurage	Southern	1880
28	8483	Shewa	Chebo & Gurage	Southern	2150
29	8485	Shewa	Chebo & Gurage	Southern	2120
30	8491	Shewa	Menagesha	4	2100
31	9058	Gojam	Debre Markos	3	2420
32	9380	Gojam	Debre Markos	3	2450
33	9424	Gojam	Debre Markos	3	2500
34	9452	Gojam	Agew Midir	3	1850
35	9456	Gojam	Debre Tabor	3	1950
36	9700	Gojam	Debre Markos	3	2400
37	9704	Gojam	Debre Markos	3	2500
38	9712	Shewa	Selale	4	2560
39	9720	Shewa	Selale	4	2880
40	9956	Shewa	Chebo & Gurage	Southern	2120

* Unusually very low elevation of Addis Ababa but referred from passport data available.

Appendix 2 Mean values of 19 morphological quantitative characters on 50 *T. steudneri* accessions.

Acc	stmt	indlgt	leflgt	lefw	stplg	stpw	flwlgt	flww	flwnum	pedlgt	petlgt	sedp	hus	flow50	mat75	plthgt	lfstra	plntwt	drym
6222	3.05	14.32	42.47	9.10	8.47	1.87	4.00	2.00	31.53	53.07	19.00	3.20	0.11	72.67	80.67	150.33	0.93	10.96	4.05
6253	2.75	14.48	42.33	8.03	8.13	1.87	3.97	1.90	25.93	46.33	12.40	4.00	0.11	77.00	87.00	138.00	0.80	15.33	6.46
6261	2.94	13.84	44.67	8.00	8.07	1.87	3.90	1.97	29.60	48.60	16.00	1.80	0.10	71.00	87.00	133.33	0.73	11.34	2.85
7620	2.61	14.26	36.60	5.33	6.67	1.60	3.80	1.87	28.33	39.67	11.20	1.93	0.10	100.00	108.00	180.53	0.73	16.21	4.86
7628	2.85	11.98	34.93	5.37	6.40	1.57	3.93	1.90	27.20	40.60	9.60	2.40	0.09	99.00	107.14	155.73	0.77	17.83	6.70
7637	3.21	11.35	35.20	5.67	5.93	1.67	4.23	1.97	29.53	30.40	12.80	1.80	0.10	103.00	111.00	161.00	1.00	18.13	7.30
7645	2.97	13.31	35.13	6.37	6.07	1.43	3.93	1.80	27.73	36.80	14.60	3.20	0.10	100.00	108.00	178.60	0.87	13.90	4.05
7652	2.87	18.02	41.73	6.10	7.67	1.63	4.00	2.00	28.73	45.20	9.87	3.27	0.16	98.33	106.33	218.33	0.90	13.69	3.99
7658	2.95	16.75	47.67	7.23	7.97	1.87	4.37	1.93	29.80	46.93	13.60	2.27	0.10	89.67	97.67	175.00	0.90	11.98	3.62
7659	2.56	12.12	36.53	5.20	5.27	1.53	4.13	1.80	27.67	35.53	12.80	2.47	0.16	101.33	109.33	132.00	0.87	15.98	6.06
7667	2.97	13.48	34.87	5.37	5.60	1.63	4.17	1.93	28.93	41.47	11.20	2.20	0.10	99.67	107.67	164.33	0.93	8.74	2.90
7671	2.94	15.42	38.73	6.17	6.07	1.80	4.17	1.90	30.93	39.00	9.80	3.53	0.10	97.00	105.00	199.60	0.97	12.43	3.66
7677	2.98	10.83	37.33	5.33	5.87	1.57	4.23	1.97	28.53	38.33	10.47	2.93	0.10	99.00	107.00	141.00	0.97	12.64	4.78
7697	2.81	10.89	35.00	5.30	5.57	1.70	4.23	1.90	24.53	28.00	10.93	2.93	0.09	101.67	109.67	135.00	0.97	17.97	6.67
7747	2.90	10.31	34.07	5.43	5.73	1.60	4.17	1.97	28.67	35.87	10.20	2.93	0.10	99.67	107.67	130.67	0.90	16.64	5.70
7779	2.80	11.73	36.60	5.50	6.00	1.53	4.20	2.00	25.80	32.07	15.47	2.60	0.10	98.33	106.33	145.33	0.93	15.72	4.64
7853	2.94	15.54	38.93	6.03	6.93	1.80	4.27	1.83	32.07	40.33	10.00	2.40	0.10	97.67	105.67	202.33	0.97	17.99	5.76
8084	2.71	12.02	40.47	7.50	8.33	1.70	4.00	1.77	29.20	39.40	10.87	3.27	0.10	78.00	87.33	114.07	0.87	10.35	3.38
8087	2.67	17.90	42.40	8.47	8.73	1.87	4.00	1.87	30.00	47.13	17.40	3.00	0.11	71.00	79.00	161.00	1.00	8.34	2.55
8329	2.85	13.03	36.87	6.93	7.20	1.83	4.10	1.80	28.67	38.53	7.93	2.80	0.10	94.33	102.33	158.33	0.77	16.76	5.03
8338	2.81	14.47	38.20	7.33	7.20	1.77	4.43	1.90	28.33	33.20	12.60	1.73	0.10	97.00	105.00	168.27	0.93	15.41	6.05
8357	2.81	17.71	37.33	6.83	7.47	1.73	4.40	1.93	29.33	41.47	10.20	2.07	0.10	97.33	105.33	219.67	0.80	8.20	2.42
8361	2.70	13.21	34.80	5.83	6.60	1.67	4.23	1.97	29.20	33.73	10.93	2.47	0.09	99.67	107.67	185.00	0.97	15.29	5.10
8450	2.85	14.88	36.20	7.37	7.17	1.67	4.30	2.00	29.27	34.13	7.93	2.07	0.10	92.67	100.67	162.00	0.80	10.29	3.73
8458	2.73	12.78	35.93	6.90	7.33	1.73	4.27	1.83	29.87	31.07	10.40	2.07	0.10	94.67	102.67	133.47	0.80	15.89	6.48
8461	2.73	16.94	41.53	6.80	7.53	1.77	4.30	1.90	26.40	30.80	11.47	2.00	0.10	86.33	97.67	180.33	0.73	15.17	5.85
8465	2.57	14.82	36.00	6.90	6.67	1.73	4.03	1.93	30.87	33.93	7.40	2.53	0.10	96.00	104.00	166.00	0.70	6.16	2.07
8483	3.21	13.87	44.80	8.30	9.27	1.90	3.80	1.80	29.87	42.07	13.60	3.00	0.11	82.00	100.00	147.67	1.00	12.89	4.92
8485	2.34	14.06	35.27	6.20	7.13	1.80	4.13	1.87	29.73	35.87	11.73	2.13	0.09	93.67	101.67	150.67	0.97	13.24	3.96
8491	2.91	12.41	40.33	7.37	7.77	1.77	3.87	1.83	29.20	39.27	13.87	2.40	0.10	83.00	91.00	138.00	0.73	9.84	2.54
9058	2.73	13.96	35.87	5.03	5.50	1.63	3.63	1.87	27.60	38.87	10.93	1.80	0.10	97.33	105.33	185.33	0.87	14.06	3.81
9380	2.87	13.90	38.47	5.87	6.37	1.70	4.13	2.00	29.53	39.80	10.27	2.20	0.10	98.33	106.33	160.00	0.97	15.22	3.79
9424	2.69	11.62	35.20	5.03	5.53	1.50	4.20	1.93	30.00	37.27	9.87	2.80	0.14	98.67	106.33	133.93	0.89	16.32	5.79
9452	2.27	13.68	27.33	7.73	8.07	2.20	4.37	2.27	33.27	33.07	22.13	2.52	0.10	122.00	131.33	200.33	1.00	21.04	10.38
9456	2.86	14.42	47.20	6.43	7.73	1.37	3.93	1.83	28.27	43.20	10.33	2.13	0.10	78.00	98.00	151.33	0.80	10.18	3.26
9700	2.42	16.16	37.87	6.40	6.77	1.63	4.13	1.90	28.93	41.40	10.67	3.13	0.11	100.33	108.33	202.67	0.93	8.86	3.11
9704	2.65	14.28	37.73	5.43	6.00	1.60	4.23	2.00	28.60	41.07	9.73	2.67	0.10	97.00	105.00	174.33	0.83	13.44	3.65
9712	2.83	14.73	39.87	5.83	6.17	1.63	4.33	2.03	27.60	41.80	11.00	2.27	0.10	98.00	106.00	180.27	1.00	18.25	6.55
9720	2.77	13.51	38.80	5.17	6.60	1.50	4.27	1.83	28.87	35.27	10.33	2.20	0.11	98.33	106.33	170.00	0.93	12.86	3.17
9956	2.92	14.57	38.47	6.90	7.53	1.83	4.43	1.93	30.20	38.47	11.00	2.73	0.11	94.00	102.00	178.13	0.90	18.49	7.51
9959	2.80	13.54	45.73	7.60	8.57	1.73	4.17	2.07	29.27	44.60	15.20	2.80	0.10	83.00	91.00	144.47	1.00	10.29	3.13
9961	2.73	18.30	38.80	7.07	7.27	1.70	4.30	1.90	29.87	38.27	10.20	2.53	0.10	97.67	105.67	221.00	1.00	13.74	6.29
9966	3.06	10.64	36.00	6.57	6.87	1.57	4.17	2.13	31.13	35.73	9.00	2.40	0.10	99.33	107.33	131.00	0.73	13.19	3.73
9970	2.77	10.62	37.27	5.87	6.53	1.77	4.07	2.03	26.40	36.33	10.67	2.80	0.10	99.00	107.00	130.33	0.83	11.62	4.42
9991	2.95	16.92	42.53	7.13	6.87	1.80	3.87	1.90	30.80	35.93	9.53	1.80	0.10	97.33	105.33	193.67	0.70	11.25	3.49
10107	2.66	12.17	36.93	6.43	7.00	1.80	3.80	1.80	29.67	33.87	11.47	2.33	0.10	99.33	107.33	138.00	0.69	8.86	1.82
10111	2.89	13.29	35.53	6.40	6.87	1.97	3.93	1.73	30.20	35.00	9.07	2.20	0.10	99.00	107.00	159.67	0.90	13.44	6.45
10125	2.93	12.42	38.87	6.13	6.83	1.70	3.80	1.83	28.67	34.60	10.93	2.53	0.09	103.00	111.00	156.67	0.90	18.25	3.79
10130	2.97	13.40	37.20	7.53	7.53	1.80	4.70	1.90	29.47	37.53	9.60	2.07	0.10	91.00	99.00	141.13	0.63	12.86	3.79
10139	2.76	14.03	34.20	6.43	7.00	1.71	4.33	1.93	30.53	37.07	10.60	2.47	0.10	99.33	107.33	171.67	0.90	18.49	9.69
Mean	2.81	13.86	38.26	6.50	6.97	1.71	4.13	1.92	29.09	38.36	11.58	2.52	0.10	94.41	103.17	162.99	0.87	13.72	4.72

Appendix 3 Cluster mean for 19 quantitative morphological characters.

CLUSTER	stmthk	indlgt	leflgt	lefwdt	stplgt	stpwdt	flwlgt	flwwdt	flwnum	pedlgt	petlgt	sedpod	hunswt	flow50	matu75	plthgt	lfstra	plntwt	drymat
1	2.83	15.01	42.18	7.75	8.08	1.81	4.03	1.89	29.39	44.25	13.76	2.86	0.11	82.23	92.37	158.11	0.89	11.36	3.98
2	2.87	13.86	38.98	6.72	7.17	1.74	4.19	1.91	29.48	36.43	10.81	2.47	0.01	94.70	103.07	163.83	0.87	15.04	4.83
3	2.76	13.93	36.21	6.60	6.96	1.77	4.12	1.91	29.47	35.97	9.62	2.35	0.10	97.52	105.52	163.91	0.80	11.01	4.37
4	2.71	13.92	37.82	5.44	6.16	1.55	4.11	1.91	28.80	39.33	10.85	2.85	0.15	99.44	107.33	161.42	0.89	15.33	5.28
5	2.83	13.19	36.78	5.82	6.32	1.65	4.15	1.93	28.47	36.92	11.13	2.38	0.01	98.16	106.17	163.72	0.89	14.88	4.86
6	2.57	14.05	37.27	7.08	7.90	1.79	4.15	2.05	30.77	38.14	16.23	2.33	0.10	100.00	114.67	175.83	0.90	15.61	6.82
Mean	2.81	13.86	38.26	6.50	6.97	1.71	4.13	1.92	29.09	38.36	11.58	2.52	0.10	94.41	103.17	162.99	0.87	13.72	4.72

Appendix 4 Summary statistics for 19 quantitative morphological characters.

	Minimum	Mean	St.Dev.	Maximum
stmthk (1):				
Group 1	2.670	2.878	0.1629	3.210
Group 2	2.340	2.812	0.1753	3.210
Group 3	2.730	2.840	0.1100	2.950
Group 4	2.420	2.809	0.1755	2.950
Group 5	2.560	2.796	0.1432	3.060
Group 6	2.270	2.270	0.0000	2.270
indlgt (2):				
Group 1	12.02	14.09	1.576	17.90
Group 2	11.35	13.66	0.9084	14.88
Group 3	16.75	16.85	0.9500E-01	16.94
Group 4	15.42	16.87	1.099	18.30
Group 5	10.31	11.37	0.7825	12.78
Group 6	13.68	13.68	0.0000	13.68
leflgt (3):				
Group 1	40.33	43.38	2.223	47.20
Group 2	34.20	36.62	1.616	39.87
Group 3	41.53	44.60	3.070	47.67
Group 4	37.33	39.42	1.808	42.53
Group 5	34.07	36.09	1.009	37.33
Group 6	27.33	27.33	0.0000	27.33
lefwdt (4):				
Group 1	6.430	7.867	0.7175	9.100
Group 2	5.030	6.161	0.7510	7.530
Group 3	6.800	7.015	0.2150	7.230
Group 4	6.030	6.533	0.4347	7.130
Group 5	5.030	5.756	0.6194	6.900
Group 6	7.730	7.730	0.0000	7.730
stplgt (5):				
Group 1	7.730	8.341	0.4596	9.270
Group 2	5.500	6.621	0.5740	7.530
Group 3	7.530	7.750	0.2200	7.970
Group 4	6.070	7.007	0.4902	7.670
Group 5	5.270	6.170	0.6743	7.330
Group 6	8.070	8.070	0.0000	8.070

Appendix 4 (Continued)

stpwdt (6):

Group 1	1.370	1.772	0.1575	1.900
Group 2	1.430	1.688	0.1188	1.970
Group 3	1.770	1.820	0.5000E-01	1.870
Group 4	1.630	1.727	0.7126E-01	1.800
Group 5	1.500	1.630	0.1041	1.800
Group 6	2.200	2.200	0.0000	2.200

flwlgt (7):

Group 1	3.800	3.960	0.9843E-01	4.170
Group 2	3.630	4.146	0.2448	4.700
Group 3	4.300	4.335	0.3500E-01	4.370
Group 4	3.870	4.163	0.1690	4.400
Group 5	3.800	4.147	0.1274	4.270
Group 6	4.370	4.370	0.0000	4.370

flwwdt (8):

Group 1	1.770	1.893	0.9487E-01	2.070
Group 2	1.730	1.904	0.7601E-01	2.030
Group 3	1.900	1.915	0.1500E-01	1.930
Group 4	1.830	1.909	0.4673E-01	2.000
Group 5	1.800	1.936	0.1012	2.130
Group 6	2.270	2.270	0.0000	2.270

flwnum (9):

Group 1	25.93	29.21	1.424	31.53
Group 2	27.20	29.00	0.9812	30.87
Group 3	26.40	28.10	1.700	29.80
Group 4	28.73	30.09	1.131	32.07
Group 5	24.53	28.23	1.996	31.13
Group 6	33.27	33.27	0.0000	33.27

pedlgt (10):

Group 1	39.27	44.85	4.221	53.07
Group 2	30.40	37.04	3.057	41.80
Group 3	30.80	38.86	8.065	46.93
Group 4	35.93	40.23	2.711	45.20
Group 5	28.00	34.41	3.006	38.33
Group 6	33.07	33.07	0.0000	33.07

Appendix 4 (Continued)

plthgt (16):

Group	1	114.1	142.0	12.66	161.00
Group	2	141.1	167.0	11.51	185.30
Group	3	175.0	177.7	2.665	180.30
Group	4	193.7	208.2	10.34	221.00
Group	5	130.3	135.1	4.698	145.30
Group	6	200.3	200.3	0.0000	200.30

lfstra (17):

Group	1	0.7300	0.8733	0.1071	1.000
Group	2	0.6300	0.8700	0.1002	1.000
Group	3	0.7300	0.8150	0.8500E-01	0.9000
Group	4	0.7000	0.8957	0.1004	1.000
Group	5	0.6900	0.8580	0.9053E-01	0.9700
Group	6	1.000	1.000	0.0000	1.000

plntwt (18):

Group	1	8.340	11.06	1.898	15.33
Group	2	6.160	14.63	3.267	18.49
Group	3	11.98	13.57	1.595	15.17
Group	4	8.200	12.31	3.074	17.99
Group	5	8.860	14.48	2.661	17.97
Group	6	21.04	21.04	0.0000	21.04

drymat (19):

Group	1	2.540	3.682	1.213	6.460
Group	2	2.070	4.950	1.821	9.690
Group	3	3.620	4.735	1.115	5.850
Group	4	2.420	4.103	1.306	6.290
Group	5	1.820	5.009	1.395	6.670
Group	6	10.38	10.38	0.0000	10.38

Appendix 6 Summary statistics for the eight agronomic characters.

	Minimum	Mean	St.Dev.	Maximum
Leflgt (1):				
Group 1	40.33	43.38	2.223	47.20
Group 2	34.07	36.94	2.526	47.67
Group 3	27.33	37.91	4.340	42.53
Lefwdt (2):				
Group 1	6.430	7.867	0.7175	9.100
Group 2	5.030	6.090	0.7529	7.530
Group 3	6.030	6.682	0.5676	7.730
Flow50 (3):				
Group 1	71.00	77.30	4.582	83.00
Group 2	86.33	97.49	3.657	103.0
Group 3	97.00	101.0	8.013	122.0
Matu75 (4):				
Group 1	79.00	89.00	6.584	100.0
Group 2	97.67	105.6	3.382	111.0
Group 3	105.0	109.1	8.450	131.3
Plntht (5):				
Group 1	114.1	142.0	12.66	161.0
Group 2	130.3	158.0	18.11	185.3
Group 3	193.7	207.2	10.01	221.0
lfstra (6):				
Group 1	0.7300	0.8733	0.1071	1.000
Group 2	0.6300	0.8645	0.9557E-01	1.000
Group 3	0.7000	0.9087	0.1001	1.000
pltwgt (7):				
Group 1	8.340	11.06	1.898	15.33
Group 2	6.160	14.52	3.025	18.49
Group 3	8.200	13.40	4.075	21.04
drymat (8):				
Group 1	2.540	3.682	1.213	6.460
Group 2	1.820	4.955	1.667	9.690
Group 3	2.420	4.887	2.409	10.38

Appendix 7 Kruskal-Wallis values for the eight agronomic characters.

```
-------TABLES ARE GROUPS (ROWS) BY ATTRIBUTE VALUES------>
       L=lower limit.....1=Mean - 1 St.Dev.....M=mean
       D=Median.....3=Mean + 1 St.Dev.....U=upper limit
       *=more than one symbol at print position
```

Leflgt (1) Kruskal-Wallis: 21.315 df: 2 Probability: 0.0000

```
   27.33            32.42            37.50            42.58            47.67
GRP +----------------+----------------+----------------+----------------+
  1                                                  L--1===D===M=======3----U
  2                          L1=======D=M========3-------------------------U
  3 L--------------------1==============M==D============3U
```

Lefwdt (2) Kruskal-Wallis: 19.753 df: 2 Probability: 0.0001

```
   5.030            6.048            7.065            8.083            9.100
GRP +----------------+----------------+----------------+----------------+
  1                          L-----------1===========M=D==========3-------U
  2 L-----1========D===M=============3-----------U
  3                   L1========D=M==========3-------U
```

Flow50 (3) Kruskal-Wallis: 21.751 df: 2 Probability: 0.0000

```
   71.00            83.75            96.50            109.3            122.0
GRP +----------------+----------------+----------------+----------------+
  1 L-1======MD=====3U
  2                        L----------1====M=D==3--U
  3                                1     LD====M==========3-----------------U
```

Matu75 (4) Kruskal-Wallis: 20.597 df: 2 Probability: 0.0000

```
   79.00            92.08            105.2            118.2            131.3
GRP +----------------+----------------+----------------+----------------+
  1 L----1======D=M========3-----U
  2                           L-----1====MD===3--U
  3                                1     LD====M===========3-----------------U
```

Appendix 7 (Continued)

```
Plntht     (5)        Kruskal-Wallis:   22.757      df:  2    Probability: 0.0000

    114.1              140.8              167.5              194.3              221.0
GRP +------------------+------------------+------------------+------------------+
  1 L---------1========M=D======3---U
  2             L------1===========MD==========3-------U
  3                                                    L--1==D===M=====3-U
```

```
lfstra     (6)        Kruskal-Wallis:   1.7193      df:  2    Probability: 0.4233

   0.6300             0.7225             0.8150             0.9075             1.000
GRP +------------------+------------------+------------------+------------------+
  1                       L------1====================DM====================3--U
  2 L--------------------------1==================M======D===========3------U
  3                 L--------------------1==================M=======D========*
```

```
pltwgt     (7)        Kruskal-Wallis:   9.0972      df:  2    Probability: 0.0106

    6.160              9.880              13.60              17.32              21.04
GRP +------------------+------------------+------------------+------------------+
  1              L---1=====D==M========3-----------U
  2 L--------------------------1===============M==D===========3---U
  3             L-----1=================D=M====================3---------------U
```

```
drymat     (8)        Kruskal-Wallis:   5.2209      df:  2    Probability: 0.0735

    1.820              3.960              6.100              8.240              10.38
GRP +------------------+------------------+------------------+------------------+
  1         *=====D===M==========3-------------U
  2 L-------------1===========D=M==============3------------------------U
  3       L1==========D========M====================3------------------------U
```

Appendix 8 The number and type of alleles in each locus for nine accessions.

ACC1 9452

ESA-1 AA:01
ESA-2 AA:03 AB:01
ESA-3 AB:04 BB:01
ESA-4 AA:03
ESA-5 AA:03 AB:01
ESA-6 AA:01
PRX-1 AB:05
PRX-2 BB:01
PRX-3 AA:01 AB:04
ESB-1 AA:03 AB:01
ESB-2 AB:04 BB:01
ESB-3 AA:01 BB:03
ESB-4 AA:03 AB:01
ESB-5 AA:04
ACP-1 AA:01 BB:04
ACP-2 AB:01 BB:04
ACP-3 AA:02

ACC2 6222

ESA-1 AA:02 AB:03
ESA-2 BB:04
ESA-3 AB:05
ESA-4 AA:01
ESA-5 AB:05
ESA-6 AA:05
PRX-1 AB:05
PRX-2 BB:05
PRX-3 AA:05
ESB-1 BB:05
ESB-2 AB:05
ESB-3 AA:01
ESB-4 AB:05
ESB-5 AA:05
ACP-1 AA:04 BB:01
ACP-2 AB:01 BB:04
ACP-3 AA:04

ACC3 7747

ESA-1 AA:05
ESA-2 AA:04
ESA-3 AB:01 BB:04
ESA-4 AA:02 AB:01
ESA-5 AB:05
ESA-6 AA:05
PRX-1 AB:05
PRX-2 BB:05
PRX-3 AA:05
ESB-1 AA:04 BB:01
ESB-2 BB:05
ESB-3 AA:02
ESB-4 AB:05
ESB-5 AA:05
ACP-1 AA:04 BB:01
ACP-2 AB:03 BB:02
ACP-3 AA:05

ACC4 9991

ESA-1 AB:03
ESA-2 AA:01 AB:03

ESA-3 BB:05
ESA-4 AA:01
ESA-5 AA:01 AB:04
ESA-6 AA:05
PRX-1 AB:05
PRX-2 BB:05
PRX-3 AA:05
ESB-1 AA:01 AB:03
ESB-2 BB:05
ESB-3 AA:01
ESB-4 AB:05
ESB-5 AA:05
ACP-1 AA:05
ACP-2 AB:04 BB:01
ACP-3 AA:04 AB:01

ACC5 10111

ESA-1 AB:04
ESA-2 AA:01 AB:03 BB:01
ESA-3 BB:05
ESA-4 AA:01
ESA-5 AB:05
ESA-6 AA:05
PRX-1 AB:05
PRX-2 BB:05
PRX-3 AA:05
ESB-1 AA:01 AB:03
ESB-2 BB:05
ESB-3 AA:01 BB:01
ESB-4 AB:05
ESB-5 AA:05
ACP-1 AA:05
ACP-2 AB:05
ACP-3 AA:04 AB:01

ACC6 8461

ESA-1 AB:05
ESA-2 AA:01 AB:04
ESA-3 BB:05
ESA-4 AA:02
ESA-5 AB:05
ESA-6 AA:05
PRX-1 AB:05
PRX-2 BB:05
PRX-3 AA:05
ESB-1 AA:01 AB:04
ESB-2 BB:05
ESB-3 AA:01 BB:01
ESB-4 AB:05
ESB-5 AA:05
ACP-1 AA:05
ACP-2 AB:05
ACP-3 AA:04 AB:01

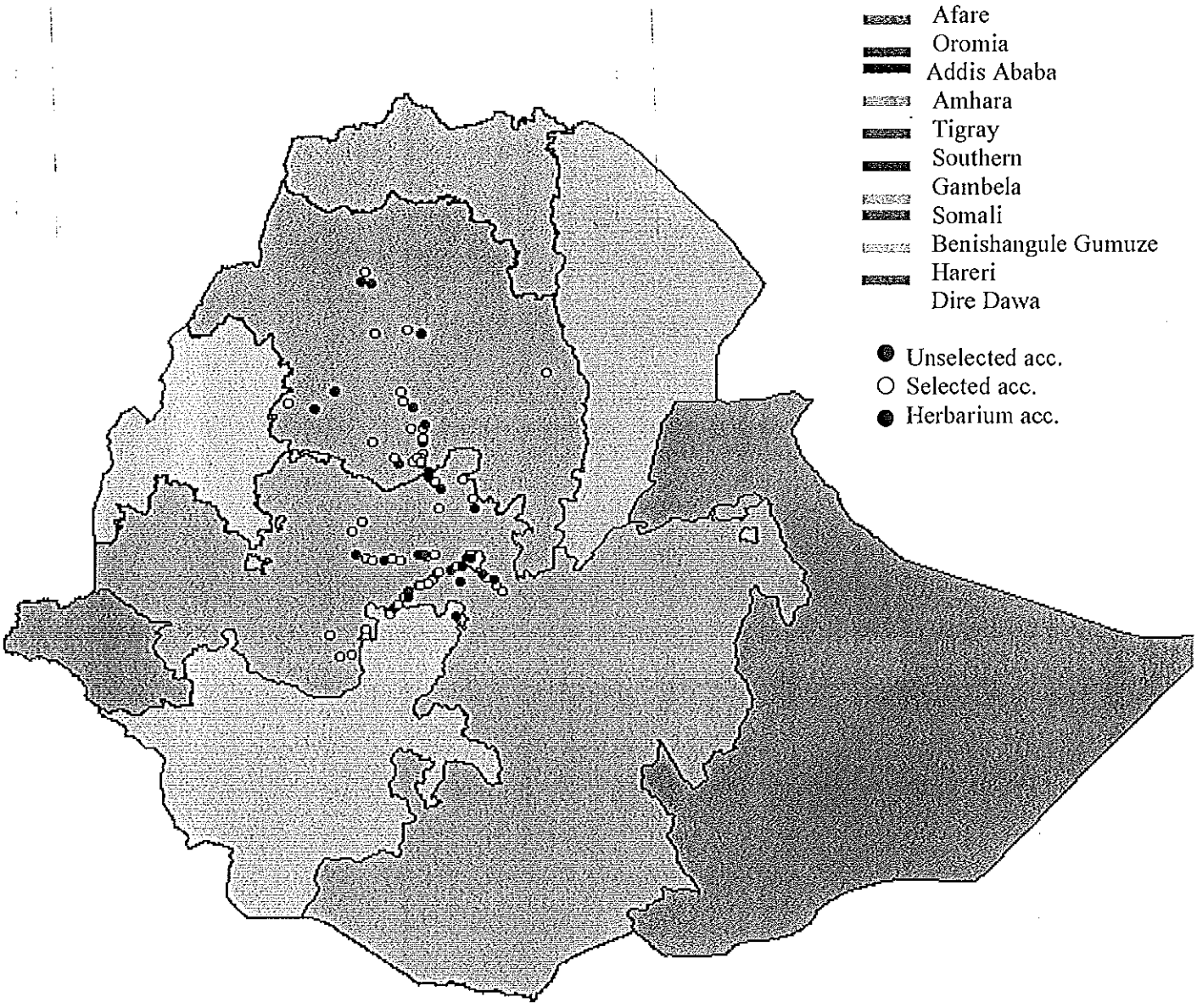
ACC7 7677

ESA-1 AA:04
ESA-2 AA:04 BB:01
ESA-3 BB:05
ESA-4 AA:02 AB:02
ESA-5 AB:05
ESA-6 AA:05

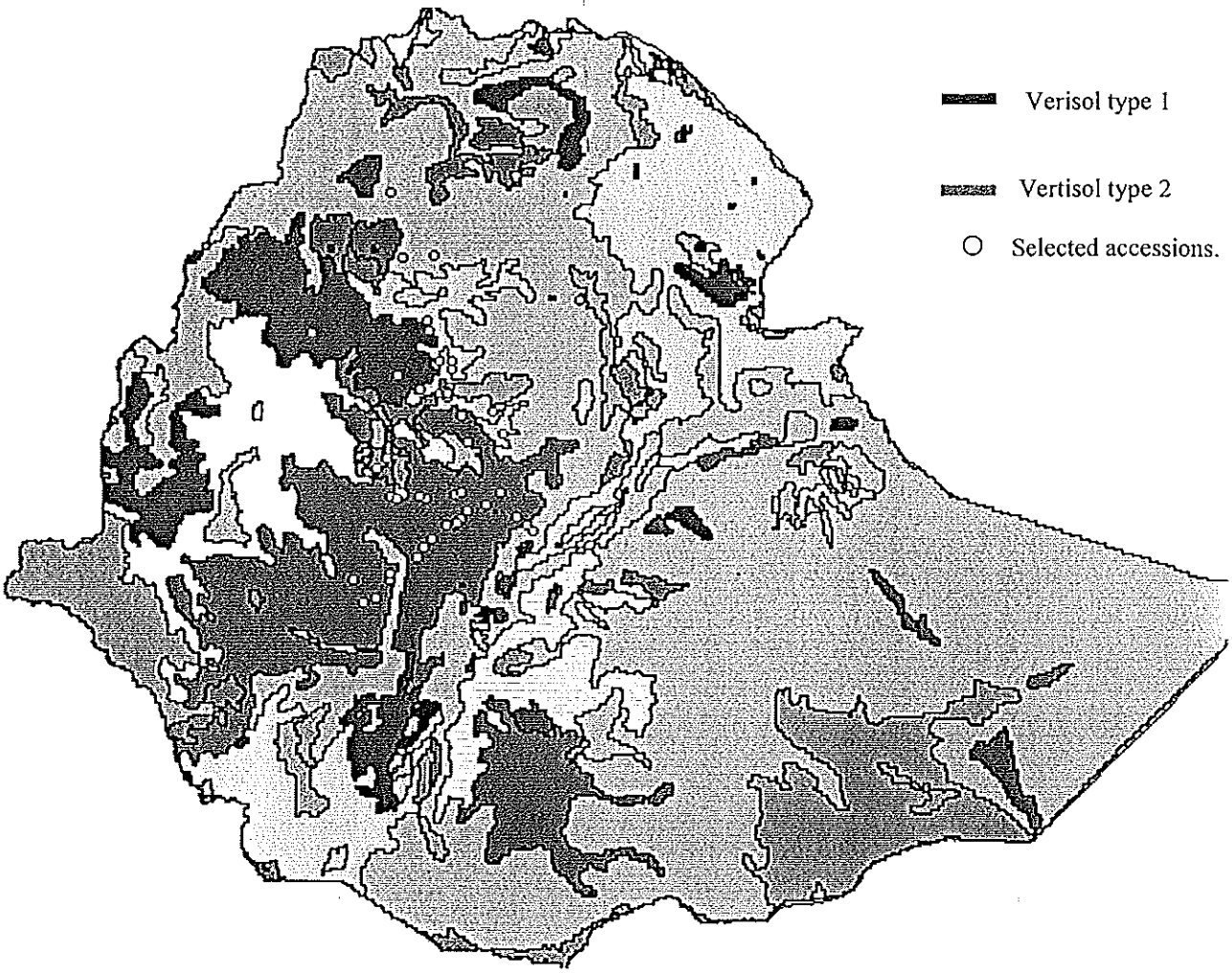
Appendix 9 Allele frequencies in accessions 1 through 9.

	Accessions								
Locus	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
ESA-1									
(N)	1	5	5	3	4	5	4	4	3
A	1.000	1.000	1.000	.500	.500	.500	1.000	.500	.833
B	.000	.000	.000	.500	.500	.500	.000	.500	.167
ESA-2									
(N)	4	4	4	4	5	5	5	4	5
A	.875	.000	1.000	.625	.500	.600	.800	.750	.000
B	.125	1.000	.000	.375	.500	.400	.200	.250	1.000
ESA-3									
(N)	5	5	5	5	5	5	5	5	4
A	.400	.500	.100	.000	.000	.000	.000	.000	.500
B	.600	.500	.900	1.000	1.000	1.000	1.000	1.000	.500
ESA-4									
(N)	3	1	3	1	1	2	4	3	3
A	1.000	1.000	.833	1.000	1.000	1.000	.750	1.000	.500
B	.000	.000	.167	.000	.000	.000	.250	.000	.500
ESA-5									
(N)	4	5	5	5	5	5	5	5	5
A	.875	.500	.500	.600	.500	.500	.500	.800	.500
B	.125	.500	.500	.400	.500	.500	.500	.200	.500
ESA-6									
(N)	1	5	5	5	5	5	5	5	5
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PRX-1									
(N)	5	5	5	5	5	5	5	5	5
A	.500	.500	.500	.500	.500	.500	.500	.500	.500
B	.500	.500	.500	.500	.500	.500	.500	.500	.500
PRX-2									
(N)	1	5	5	5	5	5	5	5	5
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PRX-3									
(N)	5	5	5	5	5	5	5	5	5
A	.600	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	.400	.000	.000	.000	.000	.000	.000	.000	.000

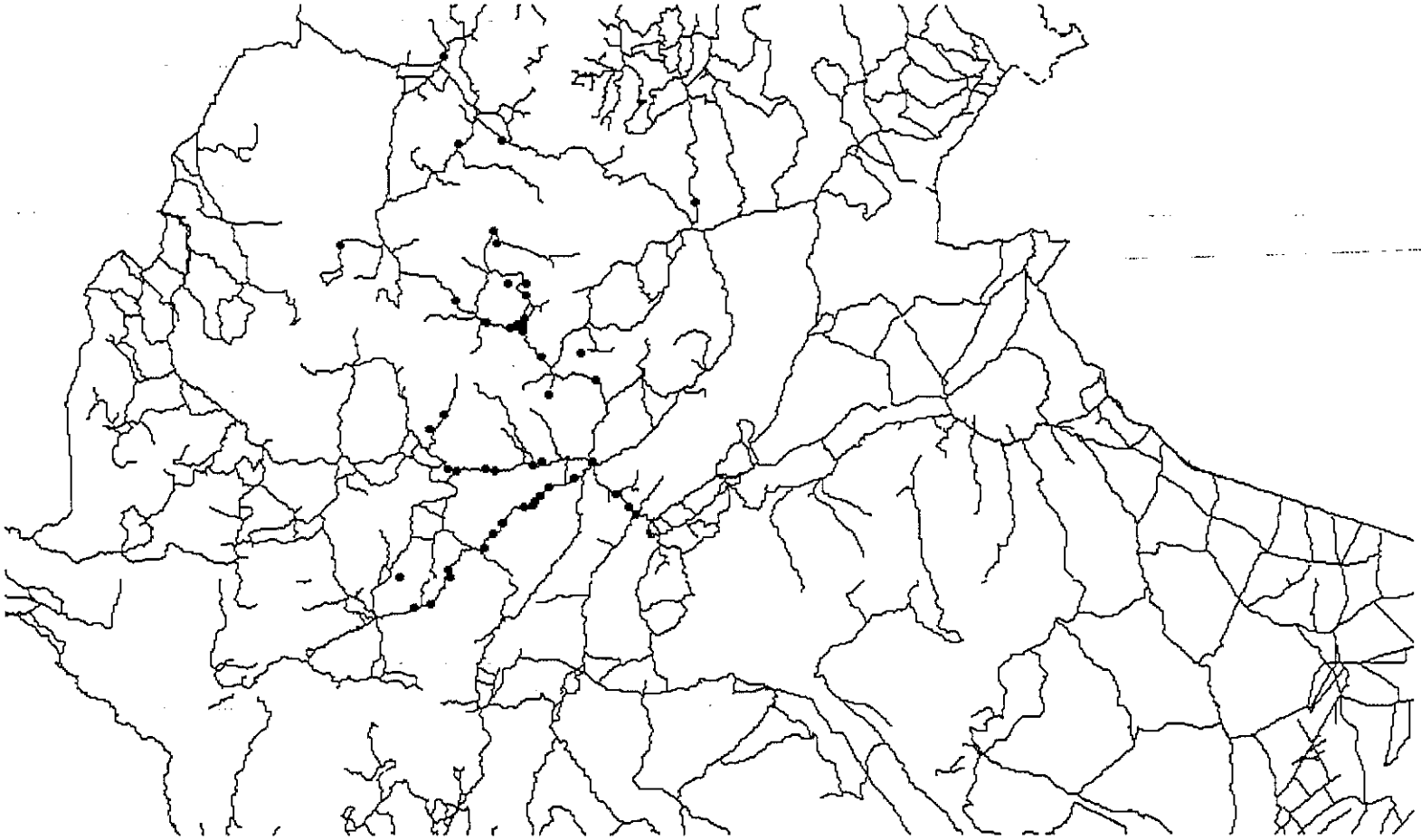
9. GIS-MAPS



Map 1 Map of Ethiopia showing collection localities of all available accessions (including herbarium collections) at ILRI genebank.



Map 2 Soil map of Ethiopia indicating that most of the accessions are collected from Vertisols type areas.



Map 3 Part of map of Ethiopia showing the main roads and the collection localities of the 50 accessions used in the study.