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MORPHOLOGICAL AND BIOCHEMICAL DIVERSITY OF GRASSPEA (*LATHYRUS SATIVUS L.*) IN ETHIOP A

A Thesis submitte ' in partial fulfilment of the requirements for the Degree of Master of Science in Biology (Applied Genetics)

By: Wuletaw Tadesse

Advisor: Prof. Endashaw Bekele

21 May 1999

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Dedicated to the Lates:

Dr. Yohannes Degago, Wollelie Melesse and Wondimagegnehu Seyoum (my colleagues)

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ABSTRACT

Fifty grasspea populations that were selected based on administrative regions and different altitude classes were used in this study. A total of 1199 entries were evaluated for 14 qualitative and quantitative morphological traits. Oxalyl diamino propanoic acid (ODAP) variation was determined for 150 entries by taking three entries from each population. Protein content was determined for the 50 grasspea populations following a standard procedure. The phenotypic variations in three enzyme systems : esterase(EST EC 3.1.1.), acid phosphatase (ACP:EC 3.1.3.2), and aspartate amino tranferase(AAT:EC 2.6.1.1) was investigated using horizontal starch gel electrophoresis for 10 populations (25 entries per population) selected from the cluster analysis result based on the morphological data.

Mean, coefficient of variations, heritability, genetic advance, correlation coefficients, cluster analysis, factor analysis and Shannon's diversity index estimate were used to estimate the morphological variation.

ANOVA (analysis of variance) result indicated that there was significant variation for the different traits within a population and between populations. The coefficient of variation was large for most traits in Gonder indicating more diversity in this region. This was further ascertained by Shannon' s diversity index where the mean diversity (H') was the highest in this region (0.65) followed by Tigray region (H' = 0.64). H at population level ranged form 0 (monomorphic) to 0.9 (polymorphic) for some traits. The over all mean diversity for Ethiopia was estimated to be H' =0.61.

Chi-square analysis also showed that there was highly significant variation for each traits (seed size, flower colour, leaflet size and pattern of testa). Some variation in altitude groups was also noted though it was not significant. Analysis of variance for mean ODAP content and protein content showed non- significant differences between populations. However, ODAP analysis from single plants showed significant variation both with in a population and between populations. Fortunately, four individual plants with low ODAP contents ranging from 0.14% to 0.18% (range with in safe level) were obtained from populations collected from different regions indicating the diversity and variation of this trait with in population and between regions. These low ODAP lines are obviously considered important for further breeding program.

Phenotypic polymorphism was observed for the three enzymes. High phenotypic polymorphism was observed for esterase. There was negative but non significant (r= -0.25) association between morphological diversity index and mean expected hetrozygosity base on isozyme data.

Key words: *Lathyrus sativus*, grasspea, lathyrism, diversity, morphological characters, ODAP, protein, isozyme, conservation.

1. INTRODUCTION

The interaction between man, other life forms and environment have resulted in innumerable patterns of variation and adaptation (Pundir and Mengesha, 1991). Several domesticates that support the world's population today are the results of natural and human selection processes.

The steadily increasing world population coupled with drought, environmental degradation, deforestation and other socio-political factors have resulted in food scarcity that nececiates the need for improved agriculture practices for increased food production. Genetic improvement of crops for drought tolerance, high yield, resistance to diseases and insect pests are the main component of agricultural improvment that can be achieved by utilizing the diverse gene sources of a given species (Melaku, 1988; Pundir and Mengesha, 1991). However, man's dependence on centres of genetic diversity for his plant and animal genetic resources are becoming very acute because of the high rate of erosion of genetic variability and structure of land race populations (Bekele, 1983). Furthermore, Melaku (1988) and Dawit (1994) showed that the valuable genetic diversity of several crops is under constant danger of being irretrievably lost due to natural calamities such as draught, replacement of the landrace by genetically uniform crop varieties and change and development in land use.

Grasspea *(Lathyrus sativus L.)* is one of the many crops that has its primary genetic diversity in Ethiopia (Vavilov, 1951; Thulin, 1983; Asfaw *et. al.*, 1994). It is a unique

crop plant, that was already in use in Neolithic times, and presently considered as a model crop for sustainable agriculture with a great future (Lambein and Kuo, 1997). The plant is unique in that it can thrive under adverse environmental conditions such as draught and flooding. It has several millennia of ecological competition under a succession of damaging agricultural and culinary practices and preferences. It is a hardy legume crop that can fix atmospheric nitrogen more efficiently than most other legumes and can grow in marginal lands with different types of soil in a wide range of altitudes. It has been used as a protein rich crop in Europe, Asia and south America. It has a multitude of local names in many languages and it is used for a multitude of purposes (Lambein and Kuo, 1997). However, excessive consumption of the seeds is associated with the incidence of a cripling disorder called neurolathyrism which is characterized by irreversible paralysis of lower limbs (Abera, 1989; Lal *et. al.*, 1986).

The association of grasspea with lathyrism is becoming a discouraging factor for grass pea production. Furthermore, the cereal based extension program coupled with the absence of improved varieties for grasspea production is another factor leading to the reduction of grass pea production and genetic resources. Hence there is a great need for germplasm collection and maintenance to promote the grasspea breeding program thereby developing high yielding but safe varieties.

Genetic diversity studies using different markers are essential to undertake germplasm collection for *ex-situ* conservation and to identify sites with high genetic diversity for *in situ* conservation (Abebe and Bjornstand, 1996; Seifu, 1997; Bekele 1983). However,

such studies were not carried out for grasspea in Ethiopia. Hence, this study was carried out with the following objectives.

1. To study the magnitude of some morphological and biochemical variability and their patterns of distribution.
2. To identify sites of high genetic diversity for *in-situ* conservation and generate information for further grasspea germplasm collection.
3. To characterize the different populations of grasspea for morphological chracters, for ODAP and total protein and identify promising genotypes for breeding purposes.

2. LITERATURE REVIEW

2.1 Origin, Distribution and Production of Grasspea

The genus Lathyrus comprises approximately 150 species distributed principally over north temperate areas but centered on the eastern Meditanean region (Goyder 1986). Lathyrus is one of the several closely related genera in the leguminosae that together form a clearly defined tribe, the *vicieae*. This tribe is characterised by the possession of leaf tendrils in the majority of its members, an unusual arrangement of vascular tissue in the stem and distinctive floral parts (Goyder, 1986). Lathyrus and Vicia, the two large genera in the tribe, each contain approximately 150 species and show parallel patterns of variation. These two genera are now separated largely on the basis of stylar hair patterns- an indumentum which is present solely on the upper surface of the style as indicative of lathyrus. In vicia it can occur on the lower surface or all round the style.

The cultivated *Lathyrus* species include: *Lathyrus annus* (F, P), *L. aphca* (F), *L. blepharicarpus* (P), *L. cicera* (F, P), *L. clymenum* (F,P), *L. gorgoni* (F), *L. hirsutus* (F), *L. latifolius* (O), *L. pratensis* (F), *L. rotundifolius* (O), *L. sativus* (P,F), *L. sylvestris* (F), *L. tingitanus* (F) and *L. tuberosus* (T) (Allkin *et.al.* 1983).(Letters in parenthesis indicate use of the species where P designates: pulse; F= fodder; O = Ornamental; and T = Tubers).

The best known species include the ornamental sweet pea (*Lathyrus odoratus*) and the food legume grasspea *(Lathyrus sativus).* Grass pea (*Lathyrus sativus L.*) belongs to the

genus Lathyrus in the tribe *vicieae* of the leguminosae family (Goyder, 1986; Perseglove, 1984). It is an annual vine closely resembling field pea (*Pisum sativum*) in growth habit, but its leaflets are long and grass shaped, hence its name grass pea. In Ethiopia, grass pea is known by different local names as: guaya in Amharic, sebere in Tgrigna, and gayu in Oromiffa (Wuletaw, *et.al* 1997a; Reda, 1989). In India and Bangladesh Lathyrus is commonly called khasari (Kaul *et al*, 1986; Lal *et al*, 1986). Its unique feature of drought tolerance makes it a golden crop for the arid regions of the world. Nevertheless, the presence of a toxic chemical in the seed which causes paralysis of the limbs when consumed excessively makes it a threatening crop (Smart, *et. al.*, 1994).

The origin of *Lathyrus sativus* is unknown (Thulin, 1983), but Purseglove (1984) postulated southern Europe and western India as centers of origin. However, Zeven and Zhukovesky (1975) placed the primary centers of diversity in the Mediterranean region. On the other hand, Vavilov (1951) and lately Asfaw, *et.al.* (1994) considered Ethiopia as primary source of diversity. According to Thulin (1983) five species: *Lathyrus pratensis, Lathyrus sphaericus, Lathyrus aphaca, Lathyrus odoratus* and *Lathyrus sativus* occur in Ethiopia but only *Lathyrus sativus* is considered to be indigenous. *Lathyrus odoratus* is cultivated for ornamental purposes.

In Ethiopia, grasspea is an important pulse crop grown in the cambisol and vertisol areas. It occupies 8.7% of the total area and 7.6% of the total production of food legumes in the country (Woldeamlak and Allelign, 1990). About 2.3 million quintals of grass pea was produced from 1988 to 1992 in a total area of 0.23 million hectares of land in the country

(Wuletaw *et. al.*, 1997c). According to the recent Ethiopian Centeral Statistical Authority report (CSA, 1998), Grasspea is the third important pulse crop after fababean and chichkpea with 142,170 ha. of production area and 1,047,440 qt. of production.

Grasspea is produced mostly in the northwest (58%), the central zone (16.3%), the northeast (12.8%) and the northern and south east region (12.9%) of Ethiopia. The distribution of the crop in Ethiopia is indicated in appendix 14. It is also a common food legume in Nepal, India and Bangladesh (Bharati, 1989; Bharati and Neupane, 1989; Kaul *et. al.*, 1986). According to Kaul *et. al.*, 1986, grass pea accounts for more than twenty five percent of area as well as production of all pulses grown in Bangladesh. Furthermore Lal, *et. al.*, (1986) indicated that *Lathyrus sativus* is the third largest pulse crop of India in area after chickpea and pigeonpea with an area of 11.9 lac hactares, production of 4.8 lac tones and productivity of 406 kg/ha.

In Ethiopia, grasspea is produced in the off-season under residual moisture. It is planted from the end of August to the beginning of October depending on the finishing and amount of the main season rainfall (Wuletaw, *et. al.*, 1997a). Harvesting is carried out in January or February after 4-5 months of planting when the leaves begin to be yellow and before pods get ripened completely to avoid loss of seeds in the field. Plants are usually up-rooted, allowed to dry in heaps in the field, then treshed and winnowed. Similar systems of cultivation is practiced in other countries (Yadave and Prasad, 1993). In Ethiopia, grass pea is usually grown as a double crop after tef or barley, the common rotation being: tef-grass pea-tef, but noug and finger millet may be used as preceding

crop. In Nepal grass pea is grown in marginal areas such as waterlogged, and lowland rice paddies which are unsuitable for good winter crops.

Grasspea is usually relay sown during November when field moisture is high at a seed rate of 30 to 40 kg/ha. Mixing the overnight soaked seeds with fresh cow dung before sowing is a common practice since it is believed that it protects the seed from birds and insects and also enhances germination (Bharati, 1986). The small seeded grasspea are believed to be harmless and are cultivated predominantly in the rice zone in a sort of relay or double cropping in which case grasspea seeds are broadcasted in a standing paddy crop, a week to ten days before harvesting (Lal *et. al.,* 1986). In all the grass pea producing countries including Ethiopia, there is no application of fertilizer, intensive ploughing and weeding for grass pea production unlike other crops.

2.2 Importance and Utilization

Lathyrus is very tolerant to drought conditions and is grown successfully in areas with an average annual precipitation of 380-650 mm (Campbell; 1989). Despite its tolerance to draught, it is not affected by excessive rain fall and can be grown on land subjected to flooding (Perseglove, 1984). Farmers grow grass pea for its ease of planting, less cost of production, its ability to grow readily side by side with a standing rice crop, its ability to maintain soil fertility through atmospheric nitrogen fixation and for its resistance to insects, diseases and weeds as compared to other crops (Lal *et. al.,* 1986; Spencer and Palmer, 1989). According to Kaul (1990), around 80% of global arid land is rain fed, and a good part of it lies in the developing world. It is believed that, lathyrogen free strains

of grasspea could have important impact on human and livestock nutrition in the resource poor countries that have vast areas of land under semi-arid conditions.

In Ethiopia, grasspea is usually prepared and consumed in the form of sauce (shiro wot), boiled grain (*nifro*), roasted grain *(kolo)* and pancake *(kita)* (Westphal, 1974; Kelbesa *et. al.,* 1994, Reda *et. al.,* 1994). Mostly, it is consumed by poor farmers and low income urban dwellers. Its ability to withstand drought makes it the only crop left when the other main crops fail. In such cases, grass pea will be the sole crop for both human and livestock and accordingly it is called an insurance poor man's crop.

2.3 Constraints of Grasspea Production

The production of grasspea is low due to its low social status, poor seed bed preparation, lack of weeding, fertilizer application and improved varieties (Adugna and Shelemew, 1994). Roy, *et. al.*, (1989), Haque and Mannan (1989) and Method (1994) indicated that neurolathyrism is the major limiting factor for grass pea production. Furthermore, crop pests (insects and diseases) also contribute for its low production (Asgelil *et. al.*, 1994; Campbell, 1989; Dereje, 1994).

2.3.1 Disease and Insect pests

Asgelil *et. al.*, (1994) and Dereje (1994) indicated that powdery mildew (*Erysiphe polygoni*), rust (*Uromyces fabae*) and wilt/root rot *(Fussarium spp.*) are the important diseases on grasspea. The first two diseases are usually severe on low lands. Aphids (*Acrithosiphon pisum*), African ball worm (*Hervicoverpa armigera*) and thrips

(*Colliothrips impurus*) are insects attacking grass pea. Aphids are by far the most yield limiting pest sometimes resulting in a total crop loss especially in the low land areas of Wondata and Zema sub districts of Gojam. Similar disease and insect pests are reported to attack grass pea in other countries (Campbell, 1989; Lal *et. al.*, 1986). Weed infestation on grasspea is very low though weeds such as *Hygrophila auriculata, Cynoden dactylon and Ghizotia scabra* are observed in some farmers fields (Asgelil *et. al.*, 1994).

2.3.2 Neurolathyrism

Considered as a whole, the leguminosae produce a range of very potent toxins in their seeds. The exploitation of legume seeds as food or feed has been fraught with considerable danger to human and animal life; possibly the most toxic are the alkaloids which act rapidly. Materials with less rapid action induce toxic amino acids (which may act as metabolic antagonists of protein amino acids), haemagglutinins, protease inhibitors, cyanogenetic glycosides and saponins (Hulse, 1976). There are also factors which can incite clinical disorders such as goiter (possibly due to effects on thyroxin metabolism) which have been found in seeds of soybeans, groundnuts, peas and phaseolus beans. Some constituents of faba beans induce favism (an allergic haemolysis) in genetically susceptible individuals (Hulse, 1991).

Excessive consumption of *Lathyrus sativus* seed for prolonged periods (3-4 months in more than 30% of daily diet) causes an irreversible crippling disorder of the lower limbs thus resulting neurolathyrism (Lal *et. al.*, 1986). It is generally described as a non

progressive deficit of the pyramidal tracts with prominent features of spasticity (Abera, 1989).

Lathyrism is one of the oldest diseases in the world. The link between over consumption of grasspea (*Lathyrus sativus)* and the susceptibility for neurolathyrism was recognized by early historians, by the British medical officers during the colonial period in India (Dwivedi, 1989) and in old encyclopedias in Europe. Historical and recent outbreaks of neurolathyrism often occurred after an environmental disaster, when the normal food crops failed due to drought, hail storms or blight and the people had to survive on the remaining crop that was *Lathyrus sativus* (Abera, 1989; Lambein and Kuo, 1997). Some of the disasters were caused by political instability or military activities.

The causative agent of lathyrism is believed to be the glutamate analogue, β-N- Oxalyl-L-2,3-Di Amino Propanoic acid (ODAP) which is found in a concentration of 0.3-2.5% fresh weight of seed (Nunn *et. al*, 1997). The safe level of ODAP depends on the thresh hold level, how much is taken up in blood and over what length of time, how much is transported into the Centeral Nervous System (CNS) and what levels are necessary to cross the blood/brain barrier (Nunn *et. al.*, 1997).

ODAP, represented by the chemical structure of COOH-CO-NH-CH_2-$HCNH_2$-COOH, occurs in the plant as two isomers, with the toxic beta-isomer predominating. The alpha-isomer is present in seeds in amounts of approximately 5% (Briggs, 1989). Various analytical methods have been developed to assay the level of this toxin in *Lathyrus*

sativus (Briggs et. al.,1997). The simplest and the most common is the Ortho-phthaldehyde (OPT) spectrophophotometric method. This method relies on the formation of a colored derivative when the hydrolysis product of ODAP is reacted with ortho-phthaldehyde. Thin-layer chromatography techniques are also simple and rapid and can be used to study several components of grasspea extracts at the same time (Birhanu *et. al.*, 1994).

2.3.2.1 Distribution of Lathyrism

The problem of lathyrism is historical in Europe and the Mediterranean region (Lambein and Kuo, 1997). However, it still remained a practically existing problem in the developing countries mainly in India, Bangladesh, Ethiopia, Pakistan and Nepal because of the biological properties of the legume and the high consumption of grasspea in these areas particularly during the period of famine.

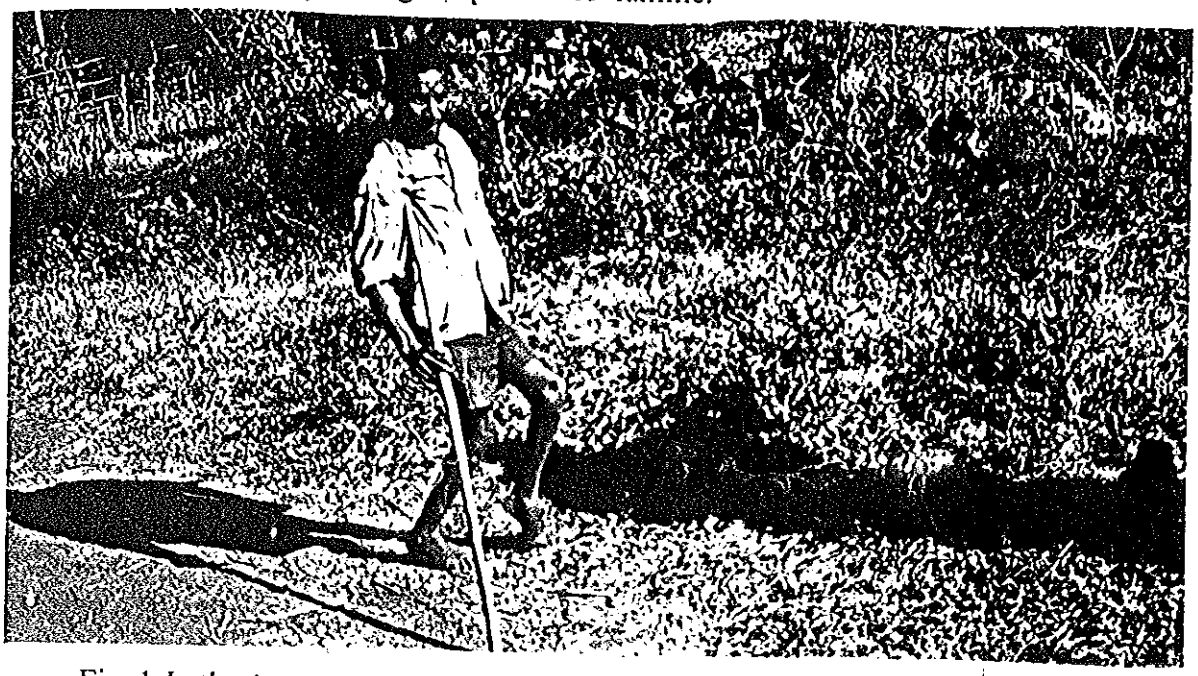


Fig. 1 *Lathyrism patient) walking with stick, Adet(Gojam) (photo by Wuletaw Tadesse 1999)*

Lathyrism and its relation to *Lathyrus sativus* has been known in Ethiopia for many years. The disease has been given descriptive ethnic names such as *sebre,* and *guaya beshita*, both implicating the leg breaking (paralytic) nature of its effect (Fig 1).

In the dictionaries relating to Ethiopian languages, Desta (1970) pointed the local folklore proverb, *Ahiyana guaya beamsaya,* which roughly translate as a donkey and the lathyrism afflicted are identical. According to Reda (1989), this saying is considered to reflect the similarity of postures of a donkey as observed to that of the cross legged spastic gait of lathyrism afflicted paraplegic.

The distribution of neurolathyrism in Ethiopia is fairly widespread over the arable part of the country with the greatest number of cases in the northern and central highland regions, particularly in the administrative regions of Eritrea, Tigray, Gondar, Gojam, Wollo and Shoa. The highest incidence of lathyrism, according to Reda *et. al.*, (1997), was recorded in the Fogera and Dembiya plains of the Gondar region and west Gojam zone. Farmers in Ethiopian Lathyrus endemic areas believe that the grass pea is most toxic when consumed in the row form, particularly if eaten with milk, for which Reda (1989) calls for further biochemical verification. The first scientific report of the occurrence of lathyrism in northern region of Eritrea was made in 1974 following the famine caused by locusts in 1946 (Cf: Reda, 1989). Although lathyrism was periodically encountered, attention from Ethiopian's was drawn to it only in 1976-77, when the Gondar college of medical sciences and Ethiopian Nutritional Institute (ENI), set out to study an unprecedented epidemic outbreak that occurred in the Dembiya and Fogera

plains of the Gondar administrative region around Lake Tana, the source of the Blue Nile, with a prevalence rates of 0.5-2.3% with similar clinical features of those seen in the rest of the world (Gebreab *et. al.*, 1978).The disease (lathyrism) causes serious psychosocial problems among the affected peasants. According to Haileyesus and Reda (1997), most of the lathyritic peoples are productive with few help from the family. Only few of them are forced to be beggars. The majority of the diseased people were engaged in farming before paralysis, many of them shifted in to less physically strenuous duties such as embroiding,) and weeding after developing their disability. Some of the male respondents had taken up church services such as deacons, priests or teachers in religious schools after their paralysis; while others are looking after cattle. Lathyritic females engaged in weaving, embroidery and basketry. Lathyrism has also a great impact in marriage. Most of the lathyritic females are single. The divorce rate in males who developed paralysis after marriage is very high (Haileyesus and Reda, 1997). In general it can be understood that lathyrism had great economical and psychosocial impacts.

2.3.2.2 Factors Affecting Neurolathyrism

The occurrence of neurolathyrism is directly related to the over consumption of *Lathyrus sativus* seed, which is precipitated by the scarcity of alternative food staffs in times of famine, following environmental disasters, when the hardy *Lathyrus sativus* survives better than other food plants. Neurolathyrism patients were mainly involved in heavy physical labour at the time of onset of the disease (Reda *et. al.*, 1994). This may imply a greater intake of food and thus a higher intake of the neurotoxin. Daily consumption of 500 g to 1000 g khasari seed which corresponds to an intake of about 5 g/day of the

neurotoxic ODAP during several weeks to several months was reported (cf: Lambein and Kuo, 1997). Considering that ODAP is a good chelator for bivalent metal ions Cu^{2+} and Zn^{2+}, it can be expected that this considerable intake of metal chelator may affect the physiological balance of these essential micro-nutrients. Epidemiological surveys in Ethiopia and Bangladesh revealed that neurolathyrism mainly strikes poor peasants involved in heavy physical labor and that the onset of the symptoms is often preceded by a diarrheal episode. These factors induce increased loss of the micro-nutrients such as copper and zinc through perspiration and stool. Both metal ions are cofactor's of numerous enzymes involved in e.g the protection against free radicals (e.g. superoxide dismutase) or in neuronal activities (e.g. glutamate dehydrogenase). An observation, that may contain a clue for understanding the higher susceptibility of young males for the toxic effects of zinc in cheleting neuro-excitatory aminoacid like ODAP, is that the loss of zinc through a single ejaculation corresponds to loosing half a litre of sweet (Lambein and Kuo, 1997). Haque (1995), compared the serum zinc level of lathyrism patients with that of healthy controls in Bangladesh. A significantly lower zinc level in patients was found as compared with the controls.

When further exploring the potential for this hypothetical role of zinc in the susceptibility for neurolathyrism, we may also look at the plant producing the neurotoxin. It was found that moderate salinity can actually have a reducing effect on the ODAP level but that drought stress can double the ODAP content in the ripe seeds (Haque *et. al.*, 1993). Also the availability of nutrients to plants grown in hydroponic solutions have sometimes dramatic effects on the toxin content of the seeds. Especially, the composition of zinc

deficiency and over supply of bivalent ion (ferro ions, Fe^{2+}) could increase the ODAP content up to four fold. ODAP has been proposed as a carrier molecule for the transport of zinc ions in the plant (Lambien and Kuo, 1997). Perhaps the result of flooding is a biological unavailability of zinc, that induced the plant to produce more ODAP. This hypothesis may explain the location of high incidence near the river banks in Bangladesh, that are flooded during the rainy season, or in the depression of the high plateau of Ethiopia, where the water from the rain keeps the soil wet and even flooded for a much longer period. Besides the varying level of neurotoxin in the seed, individual physiological variability of the people may also be part of the reason for the individual difference in susceptibility for the neurotoxin (Lambein and Kuo, 1997).

According to Reda (1989), males were affected much more than females with the ratio of 2.6:1. The onset of the disease took place below 10 years in 26.7%, 10-20 years in 27%, 21-40 years in 29.9% and above 40 years in 16.3%. Thus, 83.7% were below the age of 40 years. It is noteworthy that in females, the majority of cases had their disease onset before the age of 20 years in 69.2% as compared to 47.7 in males.

2.3.2.3 Research Efforts Undertaken to Combat Lathyrism

To combat Lathyrism, banning of grasspea production and substitution by best alternative crops have been tried by many governments of the Lathyrus endemic countries. However, it failed to be practical by farmers (Wuletaw *et. al.*, 1997b; Lal *et. al.*, 1986) since grasspea has got many advantages. Detoxification methods such as steeping of grass pea in 4 to 1 seed:water volume ratio showed that a 90% leaching out of the toxin (Pushpama,

1989). Parboiling, cooking and roasting are also important methods of detoxification (Kelbesa, *et.al*., 1994; Gedda *et. al*., 1997; Akalu *et. a*l., 1997).

Identification of zero/low ODAP grass pea strains is a long lasting and dependable solution to combat the menace of lathyrism and thereby to exploit the immense potentials of this hardy pulse. The crop improvement program at Adet, Ethiopia, identified two low toxin varieties viz; Acc.no. 46057 and Acc. no. 201513 with ODAP levels of 0.25 and 0.27%, respectively (Wuletaw, *et. al*., 1997c). In Canada, LS 8246 (Campbell, 1989) and X850002 (Campbell and Tiwari, 1997) were released for production and in India, Pusa-24 is a commercial low toxin variety (0.2%) released by the Indian Agricultural Research Institute (IARI). Line 8512 was released in Bangladesh as their first neurotoxin line. Presently low ODAP lines are also available at International Center of Agricultural Research in the Dry Areas (ICARDA) (Moniem *et. al*., 1997).

The development of biotechnology and its application in Lathyrus has resulted somaclones with ODAP content of less than 0.01% in India (Mehtha, 1997). Microbe strains which can degrade ODAP and utilize it as a sole source of carbon and nitrogen are already isolated. Mutation breeding of lathyrus has also resulted low ODAP grasspea lines (Nerker, 1972; Nerker, 1976; Waghamer *et. al*., 1997).

2.4 Genetic Diversity and Maintenance

Genetic diversity is the foundation for survival, adaptation and evolution (Nevo and Beiles, 1989). In order to breed qualities of resistance, adaptation, high yield and better

nutritive qualities in to crops, breeders need sources of variation to draw up on when required. Such sources of genetic variation are, for obvious reseasons, spoken of as "genetic resources" or "gene pools". Hawkes (1983) and Melaku (1988) described plant genetic resources as the total of genetic diversity of cultivated species and thier wild relatives, much of which are valuable to breeders. These include:

1. **Varieties (cultivars) in current use**: are varieties of recognised value and performance which have generally undergone a rather vigorous selection process by plant breeders and possess uniform characters.
2. **Obsolete commercial cultivars**: are varieties of past, and now are displaced by newer releases.
3. **'Folklore' cultivars (landraces)**: are varieties that are developed by small peasant farmers for local conditions which are generally low-input, non commercial agricultural systems. They are regarded as the real treasure house because they are the largest depository of genes for a crop ,but also are the least known scientifically.
4. **Weed races and wild species of potential value to man**: are species which are closely related to cultivated crops but not under cultivation currently. They are important sources of variability for breeding purposes with the cultivated crop varieties.
5. **Special genetic stocks**: are materials selected by man on the basis of genes for specific characterstics. It includes mutants, breeders lines, and lines with identified genes or gene combinations which are important tools for breeders.

Genetic diversity on the other hand, is usually thought of as the amount of genetic variability among individulas of a variety, population or species (Brown, 1983). Plant genetic resources with highest potential for genetic diversity are represented primarily by landraces, wild relatives of cultivated species and wild/weedy species that often contain genes for disease and pest resistance, and characters of adaptation to changing environments. Genetic diversity has been associated with environmental hetrogenity (Bekele, 1984; Hawtin *et. al.*, 1996). Accordingly, the enormous diversity of biological resources mainly crop plants in Ethiopia is accounted due to its wide ranges of agroclimatic conditions (Harlan,1968 ;Melaku, 1988; Abebe Demssie, 1992).

The importance of Ethiopia as a center of domestication and diversification of several crop species was first recognized by Vavilov some 70 years ago and later confirmed by various other scientists.The various types of terrains and the wide range of climatic and geological conditions coupled with diverse people of varied culture provide for the enormous diversity of crops in Ethiopia. Vavilov described the degree of this diversity in 1926 as follows: *"On the whole terrestrial globe, the 'Ethiopian ' center is distinguished by its diversity of forms of hulled barely, violet- grained wheat, original races of peas, peculiar races of oats and by a series of cultivated endemic plants."* Vavilov (1951) indicated that the Ethiopian region is an important primary or secondary center for some 38 crop species (Appendix 1). More recently, Zoharry (1970) identified eleven cultivated crop species as having their centers of diversity in Ethiopia.

Thulin (1983) and lately Asfaw *et. al.*, (1994) indicated that grasspea (*Lathyrus sativus)* has got a primary genetic diversity in Ethiopia. According to preliminary survey results (Melaku, 1988), high genetic diversity of grass pea is found in Gondar and Gojam while its diversity in Wollo, Tigray, Shoa, Hararghe and Eritra is medium.

2.4.1 The Role of Genetic Diversity

Despite the differences in emphasis here and there depending on crop type as well as climatic, cultural and economic differences of a country, there are underlying similarities in breeding programs (Allard, 1960). The basic objectives of most plant breeders are high yield, good quality, adaptation to soil and climatic ranges and resistance to insect pests and diseases.

The indigeneous landraces of the various crop plants species, thier wild relatives, and the wild and weedy species which form the basis of Ethipia's plant genetic resource are highly prized for their potential value as sources of important variations for crop improvement programs.

Populations of these various forms of plant species also represent sources having the greatest potential for genetic diversity and can therfore, serve as valuable means to fill the gaps that still exist in the available base of genetic diversity in the world collection of many major crop species. Among the most important traits which are believed to exist in these materials are earliness, disease and pest resistance, nutritional quality, resistance

to drought and other stress conditions and characterstics especially useful in low input agriculture (Melaku, 1988).

The existance of such genetic diversity in Ethiopia has great significance for longterm food security of the country and the rest of the world because it provides the resource base on which sustained development of high yielding and stable varieties depends. Further more, genetic diversity has another dimension than modern plant breeding for developing countries like Ethiopia. It enables farmers to reap a continous harvest of diversified food and non-food products in a more or less sustainable way to ensure survival or continuity of the societies. The traditional mixed farming systems are used to sustain productivity at the same time ensuring stable and reliable food production. This could be attributed to the crop genetic resources of farmers that show tremendous diversity and also have a wider range of adaptation. Therefore, diversity is important to small farmers so that they exercise economic sulf-sufficiency in the context of their ecological and socio-economic environment.

2.4.2 Genetic Erosion and Variation

It has been estimated that about 300,000 species of the higher plants originally extant in the world, only about 3000 (1%) are used by man. Of these, only 200 have been brought in to cultivation; and only 30 of these species supply nearly all the food consumed by the human population. About 75% of these food is provided by only eight cereal species with wheat, rice and maize accounting for 75% of this amount (Perrino, 1992).

The first narrowing of the spectrum of plant species started with the ice ages, the second with the beginning of agriculture (neolithic revolution) and the third with the beginning of scientific plant breeding which caused the displacement of traditional unimproved cultivars which had large genetic bases (Holly,1991). Genetic variation with in a plant species is a product of two kinds of interacting factors: abiotic and biotic including species characterstics like population size, mating system, mutation, migration and dispersal. Through their complex interplay and jointly with selection and random events, these factors affect the genetic composition of populations (Holly, 1991; Frankel *et. al.*, 1995; Harlen, 1971; Harlen, 1975).

The recent advances in genetic engineering also aggravates destruction of biodiversity. There are four ways in which genetic engineering destroys biodiversity in agriculture (Shiva, 1998).

1. Genetic engineering is pushing out crop diversity and narrowing the genetic base of agriculture to only a few crops. The global trends of the growth of genetically engineered crops is as follows: Soyabean (40%), corn (25%), tobacco (13%), cotton (11%), conola (10%), tomato (1%) and potato (1%).
2. Genetic engineering is accelerating the expansion of monocultures of these crops.
3. Genetic engineering is leading to the destruction of on-farm biodiversity through the use of broad spectrum herbicides like Round-up associated with Round-up Ready resistant crops

4. Genetic engineering is threathening the survival of species especially through the ecologically impact of bt. Crops, Bacillus theringensis engeneered crops

In Ethiopia, the exsiting broad range of genetic diversity particularly that of primitive and wild gene pools, is being rapidly depleted, displaced or abandoned due to causes that are many and complicated (Bekele, 1983; Melaku, 1988; Abebe Demssie, 1992). Various factors have interplayed in posing such threat whch is progressing at an alarming rate. The most important ones include displacement of indigeneous landraces by new, genetically uniform varieties and due to changes and development in agriculture or land use. Problems like drought, various factors related to the national breeding program and other socio-political factors also directly or indirectly contribute to persistent loss of genetic resources, narrowing dawn of of gentic base, or even genetic wip out (Melaku, 1988).

The association of grasspea with lathyrism coupled with the cereal based extension program discourages grasspea production and accordingly farmers change their land use system which inturn leads to genetic erosion especially in the areas where grasspea production is banned.

This hazard should be prevented. Some plant materials may not appeal now for our present requirements but may prove to be paramount importance in the future. Plant material has to be therefore, collected while it exists for utilization in the present and future breeding programs.

2.4.3 Genetic Maintenance

Genetic conservation has arisen as a solution to some of the problems caused by man in his social and agricultural relationship with the environment. Unwise exploitation of nature has caused an irreversible loss of variability and become the major cause of world-wide genetic erosion. The seriousness and rapid expansion of the problem has created a universal need to collect and conserve genotypes that would no longer be available if not conserved today.

The loss of crop genetic resources in the developing countries was so crucial that it attracted the attention of many scientists and nations which led to the establishment of the International Board for Plant Genetic Resources (IBPGR) in 1974 so as to coordinate global network of gene banks and provide plant breeders with diverse genetic resources necessary for sustained food production.

Although it is possible to conserve all available gene resources, a practical and successful strategy has been to identify germplasm categories and from each of them to collect and conserve a representative sample. Methods for maintenance of genetic resources vary according to several factors, including the species, their geographical distribution, breeding system and seed behaviour (Perrino, 1992). However, two main methods have so far been identified.

Ex-situ conservation: This is conservation in an artificial habitat or in a habitat different from the original one. Populations, indviduals, tissus and organs, cells and DNAs can be conserved in genebanks

In-situ conservation: conservation of landraces and wild relatives in their natural habitats in areas where genetic diversity exists and where wild/weedy forms are present, often hybridizing with the cultivated types, represents a vital componenet of preserving diversity.

Both of the above strategies have thier own drawbacks. Although a number of gene banks have been established in many countries of the world, that alone does not represent adequate conservation measure to the useful crop plants as it has got the following drawbacks. Firstly, conserving germplasms in gene banks is associated with high cost which is not affordable by developing countries. Secondly, there is loss of diversity in gene banks due to degeneration of material in storage and accidental losses among others. Further more, when materials are stored under frozen condition, there is no evolutionary process going on for about hundred years or so which inturn arrests evolution and thus generation of variation.

The most difficult problem associated with *in-situ* conservation is the selection of optimal ecosystems where the different species with economic importance could be best maintained, and to establish a sufficient number of reserves which represent as much of the naturally occuring diversity as possible (Holly, 1991). Further more, keeping

germplasm where it has evolved will not make materials readily available for breeders as they may not be well described and evaluated. It seems also to turn the clock back and condemn the poorer farmers to grow a mixture of varieties, some of them perhaps high yielding types but many of them not. Hence the practicallity and sustainability of this strategy is questionable since it will not encourage the farmer to use mixtures of landraces while there are improved high yielding varieties available to him.

Many scientists have pointed out that combination of the two approaches is important for sustainable conservation of genetic resources (Brush, 1989; Plucknett *et. al* 1987; Altieri and Merrick, 1987; Melaku Worede, 1990). Incomplete collection in gene banks, loss with in collections, evolutionary processes in cropping systems and budgetery constraints can be considered as important reasons for this (Brush, 1989).

The collection and conservation of crop genetic resources coordinated under the auspices of Food and Agriculture Organization (FAO) and IBPGR were initially based on the understanding that germplasm is a common heritage. However, recently the issue of management and use of crop genetic resources has entered the political arena as germplasm started being stored in gene banks for longterm conservation. Ownership of genes and patenting are still an international debate with no clear cut answer about who has to benefit out of conserving the germplasm resources (Plucknett *et. al.* 1987; Fowler and Mooney 1990; Shiva, 1998). Landraces are property of farmers and belong to them. However, the diversity of most crop genetic resources is in the hands of industrialized nations and are being exploited by multinational corporations at the expense of the

developing nations (Bayush Tsegaye, 1991)

Conservation of seeds in gene bank is the most common method in Ethiopia (Seifu, 1997; Abebe 1992; Melaku, 1988, Regassa, 1986). To avoid the rapid genetic erosion, the plant genetic resource center of Ethiopia follows two strategies in germplasm collection for *exsitu* conservation, namely general and specific/pointed missions (Dawit, 1994). The general collection expedition is to tap the maximum genetic diversity in different crops growing in the region and maturing more or less at the same time. The specific or pointed type is undertaken to collect diversity in a particular crop, group of crops or for particular characteristics in a species which is mainly practiced in collaboration with other institutions.

At present, the center holds about 50,150 accessions of mainly cereals and millet (75.30%), pulses (9.50%), oil crops (7.92%), stimulants (2.41 %), spices (2.07%), root crops (0.52%), medicinal plants (0.29%) and others (1.99%) (Abebe Demssie, 1992). Lathyrus has been collected as a result of the general collecting expeditions to over come the progressive genetic erosion. A total of 282 grasspea accessions are found in PGRC/E which is 6% of the total holding of pulse crops in the center (Dawit, *et. al.*, 1994).

2.5 Genetic Markers

The study of genetic diversity and structure of species of interset has been greatly facilitated by the use of morphological, biochemical and molecular marker types (Bekele, 1983; Seifu, 1997; Abebe and Bjornstad, 1996; Abebe & Bjornstnd, 1997).

2.5.1 Morphological Markers

Highly heritable morphological traits such as leaf colour, flower colour, seed colour, seed size etc. were among the earliest genetic markers used in scientific investigations and are still in use in germplasm management (Bretting and Widrlechner, 1995). Vavilov's determination of centers of diversity for various crop species were based on his extensive field studies and observation of phenotypic traits.

Through the application of multivariate methods, it has been possible to analyse and describe the variation in *L. sativus* and to assess the relationships of *L.sativus* and some species in section *Lathyrus*. *Lathyrus sativus* shows great morphological variation, especially in vegetative characters such as leaf length, while floral characters are much less variable (Jackson & Yunus, 1984). This array of variation is undoubtedly the result of geographical separation as well as selection by man. The pattern of variation in *Lathyrus sativus* based on flower color and seed coat color agrees with that described by Vavilov (1951), who also noted that forms of *Lathyrus sativus* with white flowers and white seeds were highly selected and recessive. Such forms were typical of a more western distribution around the mediterranean basin. One can postulate that the forms with blue flowers and speckled seeds are more primitive, and the pattern of variation found in *L. sativus* is like that found in other pulses such as lentils and broad beans (Jackson & Yunus, 1984).

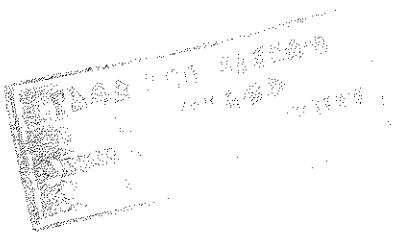
Genetic variation studies using morphological traits which are of interest to the breeder's are important to speed up the breeding program. They are also inexpensive, simple and

rapid to score. However, morphologcal markers have also weaknesses such as many of the traits are controlled by many genes, they are influenced by environment, the phenotype of most morphological markers can only be determined at the whole plant level; allele frequencies at morphological marker are much lower; alleles at morphological loci interact in a dominant- recessive manner that limits the identification of hetrozygous genotypes, and require growing of plants to a suitable stage before certain characters can be scored (Powell, 1992; Seifu, 1997; Wendel and Weeden, 1990).

2.5.2 Mollecular Markers

The relative ease with which DNA molecules can currently be cloned allied to the availability of a wide range of restriction endonucleases has allowed a much greater portion of the plant genome to be assayed for genetic markers.

The development of the polymerase chain reaction (PCR) has revolutionized the analysis of nucleotide sequence variability . The procedure for the identification of polymorphism in plants based on PCR is described by Williams *et. al.*, (1990). Both RAPD (Randomly Amplified Polymorphic DNA) and RFLPs are being used extensively for the study of genetic diversity. DNA markers in general are considered as ideal for diversity study and enabled to overcome the short comes of other markers, eventhough, they are expensive and have their own limitations Powel, 1992).



2.5.3 Biochemical Markers

The most commonly used biochemical markers are isozymes (Wendle and Weeden, 1990). Isozymes are defined as variants of the same enzymes having identical or similar functions, but differing in electrophoretic mobility (Markert and Moller, 1959). They are revealed when tissue extracts are subjected to electrophoresis in various types of gels and subsequently immersed in solutions containing enzyme specific stains. Electrophoretic separation of complex mixtures of proteins can be accomplished in several types of support media, including starch gel electrophoresis (SGE), Polyaclyramide gel electrophoresis (PAGE) and agarose gels, and cellulose acetate membranes (Wendel and Weeden 1990). The two most widely used systems are starch and polyacrylamide gel electrophoresis. Isoelectric focussing (IEF) is a further protein electrophoretc technique with a high resolution (Radola, 1980).

Despite the greater resolving power of PAGE and IEF, the relative simplicity of starch gel preparation ensures that SGE is still a widely used and effective separation system. In addition, SGE does not involve the use of toxic material where as the acrylamide used for PAGE and IEF is a neurotoxin (Wendel and Weeden,1990; Powell,1992). Furthermore, starch gel systems can be easily replicated to allow the simultaneous evaluation of a range of enzyme systems.

The ability to observe allelic variation at isozyme loci has revolutionized research in the fields of biochemical genetics, population genetics and evolution. This variation, called allozymic polymorphism, has been used in plants to examine genetic processes at every

stage of the life cycle and to ascertain genetic diversity in all major crops as well as many other species (Wendel and Weeden, 1990). Isozymes are qualitative markers that have been assayed frequently because they are relatively numerous, the assay is inexpensive, and rapid, and generally they are unaffected by growing conditions (Beer *et. al.*, 1993; Seifu, 1997). Furthermore, they are frequenly polymorphic, exhibit codominance and simple Mendelian inheritance (Wendel and Weeden, 1990; Brown, 1978). An exception to codominance is the presence of *null alleles,* alleles that are no longer transcribed or that code for defective polypeptides lacking enzymatic activity. The inheritance of allozymes in a Mendelian fashion allows one to ascertain allelic frequencies for populations of plants, species etc. (Seifu, 1997). In addition to the study of genetic diversity and evolution, isozymes are also important for plant breeding as follows: Screening of germplasms for new sources of variation, identification of varieties, marking of monogenic traits, the study of gene introgression and manipulation of quantitative traits and plant mating systems (Tanksley and Orton, 1983; Brown *et. a*l., 1989; Wendel and Weeden, 1990). Despite the redundency of genetic code and the possibility of amino acid substitutions not affecting the overall charge on the protein, changes in the electrophoretic mobility of enzymes provide an extremely useful method of evaluating genetic differences among groups. The more recent findings of introns with in the structural genes and the existence of multigen families for many proteins have not seriously undermined the ability of isozyme polymorphism to serve as a direct measure of DNA sequence variation with in and among genomes.

2.6 Correlation Between Markers

Price *et. al,* (1984) has observed that estimates of population differentiation based on isozymes and quantitative traits were strongly correlated in a natural populations of *Avena barbata* (selfing species) while negative correlation was observed in an out breeding species, *Clarkiawilliam soni.* Significant positive associations between RFLPs and allozymes genetic distances in rape seed was reported (Becker *et. al.*, 1995). Similar positive correlation was also obtained between morphological and biochemical markers (Cross *et. al.,* 1992) and morphological and isozyme diversity (Brown and Mundey, 1982) in barely. Furthermore, significant associations were found between particular isozyme genotype and every trait analyzed in soyabean (Suarez *et. al.,* 1991).

Stauab *et. al.* (1997) indicated that although dendrograms derived from cluster analysis using species variation at marker loci were disimilar, these desparities were consistent with differences in the pedigrees and/or other information (e.g. morphological) known about each accession and species in cucumis. Negative correlation between morphological markers and allozyme was reported in durum wheat (Seifu 1997) and in Brassicinae (Takahata and Hinta, 1992). Positive but non significant correlation between morphological and biochemical markers was obtained in barley (Abebe and Bjornstand, 1997; Bekele, 1984). In general the lack of consistent agreement between different markers indicated the poor predictability of variations that can be revealed by one class of markers based on the others. Accordingly it is difficult to exclusively select and apply only one type of marker for diversity study. However, due to its

simplicity and cost effevctiveness, isozyme electrophoresis has been the most commonly used to measure genetic diversity and structure of plant populations (Hamrick and Godt, 1997).

3. MATERIALS AND METHODS

3.1 Morphological Study

3.1.1 Description of the Study Area

This study was conducted at Adet Research Center which is located at 37° 29' E and 11° 16 ' N in the Amhara National Regional State. Adet is 45 km from Bahir Dar along the main road from Bahir Dar to Addis Ababa via Mota. It is located at 2240 m a.s.l. The experimental site is dominantly vertisol which is ideal for grasspea production, as a result of which the grasspea research in Ethiopia is centered at this research center.

Soil physico-chemical characters of the experimental site is indicated in Appendix 2

3.1.2 Experimental Procedures

A total of 50 grasspea populations including one local check (acc.no .208449) were used for this study. The materials were obtained from plant genetic resource center of Ethiopia (PGRC/E). Selection of the populations for this study was based on altitude, and administrative region. Table 1 shows the list, origin and altitude of the populations used for this study. The regions from which the populations are collected are indicated in appendix 13.

Table 1. Plant genetic resource center (PGRC/E) acc no., administrative region, county and altitude of the collecting sites of the populations

No.	Acc.no.	Region	County	Altitude(m)
1	46003	Shoa	Yerer	2050
2	46008	Shoa	Muketurie	2240
3	46012	Gojam	Mota	2550
4	46016	Shoa	Sayadibir	2700
5	46020	Shoa	Ambo	2235
6	46023	Shoa	Menagesha	2500
7	46024	Shoa	Kara	2460
8	46027	Shoa	Ginchi	2420
9	46030	Gojam	Enebsie	2300
10	46033	Gondar	Este	2330
11	46034	Wollo	Wogeltena	2090
12	46035	Wollo	Wogeltena	2375
13	46042	Gojam	Debrework	2540
14	46044	Gojam	Shebelberenta	2410
15	46049	Gojam	Dejen	2490
16	46050	Gojam	Felegebrhan	2700
17	46073	Gojam	Yetnora	2420
18	46099	Wollo	Kalu	1880
19	46100	Wollo	Bistima	2000
20	46106	Wollo	Wodere	1950
21	46110	Wollega	Kelem	1600
22	46111	Wollo	Sulula	1700
23	207493	Gondar	Kemkem	2400
24	207496	Tigray	Didiba	2200
25	207497	Tigray	GentaAfeshum	2200
26	207499	Gondar	Gondar	2600
27	207566	Tigray	Hintalo	2150
28	207567	Tigray	Laymaychew	2100
29	208449	Gojam	Adet	2300
30	211511	Wollega	Nejo	1740
31	215247	Wollo	Gobalafto	1900
32	219945	Tigray	Adiabeiti	1870
33	219946	Tigray	Laymaichew	2080

34	219949	Tigray	Adiabeiti	2150
35	219950	Tigray	Adwa	2230
36	219952	Tigray	Naendre	1700
37	220118	Ertrea	Mendefera	1980
38	226001	Wollo	Debresina	2400
39	226006	Wollo	Kelela	2500
40	226010	Wollo	Legambo	2640
41	223219	Tigray	Tserai	1930
42	226014	Gondar	Este	2645
43	226019	Gojam	Bahirdar	1685
44	228495	Gojam	Awbel	2400
45	236711	Gojam	Damot	1840
46	46107	Gondar	Maksegnit	1950
47	236686	Gojam	Mertolemariam	2555
48	436705	Gondar	Woreta	1800
49	236708	Gondar	Dabat	2730
50	236701	Gojam	TisAbay	1700

The experiment was laid out in randomized complete block design with three replications in single row of five m lengths. The spacing between plants was 0.5 m and the spacing between rows (populations) and replications were 1 & 2 m, respectively. Eight individual plants were tagged and used for evaluation in each row. Twenty-four individuals per population, (except 23 for one population), and a total of 1199 individual plants were evaluated for the following agronomic characters using international board for plant genetic research (IBPGR) descriptor.

1. **leaf let size**: this was measured using three scales where 3= small, 5= medium and 7= large.
2. **Days to flowering:** the number of days from planting up to the appearance of the first flower
3. **Days to maturity**: the number of days from planting up to physiological maturity. This is at the time when the color of leaves and pods changed from green to yellow.
4. **Flower colour**: this was scored at the time of flowering. Red ,pink and blue flower colours were recorded as 1, 2 & 3 , respectively.
5. **Plant height**: the height of the plant from the soil surface to the upper most leaf measured at maturity in cm.
6. **Number of primary branches**: The actual count of the number of primary branches on the main stem at harvest.
7. **Number of pods/plant**: the actual count of the number of pods/plant at harvest.
8. **Number of seeds/pod:** the total number of seeds per plant divided by the total number of pods per plant.
9. **Seed size:** this was rated as 1 for small, 2 for medium and 3 for large seeds by observing the total harvested seeds per plant.
10. **Hundred seed weights (100 seed weight)**: 100 seeds were randomly taken from each plant and weighed using sensitive balance in g.
11. **Seed color:** was observed from harvested seed of each individual plant and recorded as 1 for olive, 2 for gray, 3 for brown and 4 for black.

12. **Seed yield per plant**: the dried weight of seeds from each individual plant measured in g.

13. **Dry matter weight**: the harvested plant was dried in open air untill it reached constant weight.The final weight is dry matter weight in g.

14. **Harvest index:** the ratio of seed yield per plant to the dry matter weight per plant multiplied by 100.

3.2 Biochemical Studies

3.2.1 ODAP analyses

A total of 150 individual plants, three from each of the 50 populations, were used for this study. The three plants per population were selected based on the morphological study.

Each of the sample seeds were grind by A-10 analytical mill for 2 minutes. Then extraction was made by taking 80 mg seed powder and dissolving by 8 ml distilled water which was then vortexed for 30 seconds in screw cap test tube. The mixture was sonicated at 45-55°c for 1 hour. Then the solution was centrifuged for 5 minutes. Hydrolysis was carried out by taking 100 microlitre of the filtrate in a test tube to which 200 microlitre 3 N KOH was added. The mixture was kept in a boiling water bath for about 30 minutes. Then after cooling, the base was neutralized by adding 200 micro litre 3 N HCl. The total volume was made 1 ml with distilled water. Aduct formation was made by taking 1ml of the hydrolysis product and 2ml of OPT reagent, and the mixture was let to stand for about 30 minutes. Absorbance of the aduct was measured at h max 476 NM against a reagent blank. The standard sample used was dl-2,3- diamino

propanoic acid. ODAP of the samples was determined using the standard curve, absorbance, concentration and slope relation (Rao *et. al.*, 1964) . The slope was dtermined from the standard curve (Appendix 15).

3.2.2 Protein Analysis

Seeds from fifty grass pea populations, which were studuied for morphological diversity at Adet were used for this study. Protein analysis was carried out using kjeldahl apparatus as per the standard protocol for nitrogen analysis. Percentage N was multiplied by 6.25 to get the protein content for each grass pea samples.

3.2.3 Isozyme Study

Isozyme analysis was carried on ten accessions of grass pea selected from five clusters based on morphological diversity, i.e. two populations from each cluster were used. The selection of the two populations from each cluster was based on diversity index, source region, and ODAP content. Twenty five seedlings per accession were studied. Seven and three days old leaf samples were compared for extraction and better resolution. The three day old leaf gave better resolution and hence extraction was made from three days old leaves. Crude extracts were prepared by macerating leaves in two drops of extraction buffer (0.05M sodium phosphate pH 7.0, plus 0.2M 2-mercaptoethanol). The perspex extraction trays were kept on crushed ice during maceration to prevent denaturation of the enzymes. Extracts were absorbed on to wicks made from Whatman 3MM chromatography paper. Horizontal electrophoresis was carried out in 12% starch gels. Two buffer systems were used:

1. **Lithium borate buffer pH 8.3**: the gel buffer for this system contains 5.4 g tris base and 1.28 g anhydrous citric acid. The electrode buffer contains 1.2 g lithium hydroxide and 11.9 g boric acid (pH 8.3).

2. **Histidine tris citrate buffer PH 7.5:** the gel buffer for this system contains 8.3 g histidine-HCl and 0.03 g EDTA. The electrode buffer for this system contains 15.1g tris-base and 7.3 g citric acid. Twenty five samples were run on each gel plus two wicks dyed with bromophenol blue to act as a marker control. Electrophoresis was carried out at 4°c with a constant current of 70 mili ampere (250 volts) for lithium borate gels and 50 mili ampere (200 volts) for tris-citrate gels. Gels were run 8 cm approximately within 4-5 hours.

Three enzyme systems were selected for detailed analysis after a preliminary survey of five enzymes (ACP:EC 3.1.3.2, AAT: EC 2.6.1.1, EST:EC 3.1.1, PRX:EC 1.11.1.7, and LAP: EC 3.4.11.1) since they gave consistent results with this species. The three enzymes analysed were: esterase **(EST)**, aspartate amino transferase **(AAT)**, and acid phosphatase**(ACP)**. Buffer system 1was used for **EST** and **AAT**,while buffer system 2 was used for **ACP**.

In the first gel system, the gel was cut in to three slices. The top slice was discarded since most enzymes did not stain well on it. For buffer system 1, the second slice was used for **EST** and the third slice for **AAT**. For buffer system 2, the second slice was used for **ACP**.

The following staining recipes were used following protocols developed by Wendel & Weeden, 1990; Chamberlin, 1998.

For ACP: 50 ml 0.4 M sodium acetate buffer PH 5.0 which was used to pre-soak the gel for 20 minutes at 4°c, 50 miligram beta napthyl acid phosphate, 50 miligram fast blac kk salt, and 0.5 ml 10%$MgCl_2$.

For AAT: 50 ml 0.1 M tris-HCl (PH 8.5), 18 mg alpha ketoglutaric acid, 65 mg DL-aspartic acid, 250 mg PVP, 50 mg disodium EDTA, 710 mg Na_2HPO_4 and 200 mg fast blue BB salt; and

For EST: 20 ml distilled water, 20 ml 0.2 M NaH_2PO_4, 10 ml 0.2 M Na_2HPO_4, 2 ml 1% α-naphtyl acetate and 125 mg fast blue BB salt and 1 ml acetate were used.

Variation in banding patterns was determined by the migration from the origin towards the anode. Isozyme zones were designated to define the general area on the zymograme with in which the bands migrated. The zones were numbered from the fastest to the slowest migration from the point of insertion of the wicks in the gel. Scoring was made for those bands which were clearly visible. An assessment of isozyme phenotypic polymorphism was made using the overall banding patterns. Phenotypic polymorphism, genetic distance, degree of differentiation (FST) and hetrozygosity were determined using Biosys software (Nei, 1978). A tentative genetic interpretations of the banding patterns was made based on the reported structure of each enzyme in different plant species (Wendel and Weeden, 1990) and particularly in related genera such as pisum, lens and vicia, where the information was available.

3.3. Statistical Analyses

All the crop based data collected were subjected to analysis of variance (ANOVA) using the MSTAT C computer programme. Cluster analyses were computed based on quantitative characters, qualitative morphological characters and both qualitative and quantitative morphological characters using SAS software (SAS institute, 1985). Simple correlation coefficients between all possible traits were computed for each altitude groups and regions. Genetic and phenotypic correlations were calculated as follows: genotypic correlation = COV xy/ $\sqrt{}$(S^2gx*S^2gy) where COV xy is the genetic covariance between two traits, S^2gx and S^2gy are the genetic covariances of the two traits. Phenotypic correlation = COV xy/ $\sqrt{}$(S^2px*S^2py) where COV xy is the phenotypic covariance between two traits, S^2px and S^2py are the phenotypic covariances of the two traits. Cov xy for each pair of traits was computed from the analysis of covariance in a similar manner as the analysis of variance. 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 and 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).

Factor analysis was carried out using SPSS software. Diversity of morphological characters was measured using Shanon's diversity index , $H_{sj} = - P_i \ln P_i$, where P_i is the relative frequency in the i^{th} category of the j^{th} trait. This index was used to study diversity in wheat (Jain *et. al.*, 1975), and bareley (Abebe and Bjornstad , 1996; Bekele, 1983; Negassa, 1985). The average diversity (H') over K traits was estmated as $H' = h_{sj}/K$. Since different numbers of phenotypic classes were recognised for the five characters, H was standardized by converting it to the relative index, H' = H/Hmax, where Hmax = (ln n). H' estimates the proportion of the theoretically possible maximum variation existing in a population (Hennink and Zeven, 1991). According to Pielou (1966), the relative index is always between one and zero. It becomes one when all phenotypic classes are represented by an equal proportion and it becomes zero when all entries with in a character belong to one phenotypic class. Chisquare analysis was carried out for each trait using regions as classifying variables. Allele polymorphism and genetic distance were determined using biosys software (Nei, 1978).

4. RESULTS AND DISCUSSION

4.1 Morphological Diversity

4.1.1 Mean, Range and Coefficient of Variation for Quantitative Characters

Mean, range and coefficient of variation in agronomic traits are widely used to determine variations with in and between populations (Endashaw Bekele, 1996, Belay, 1997, Sharma, *et.al*, 1995, Jaradat,1991).

Summary statistics (mean, and coefficient of variations) over the entire individuals of the 50 grasspea populations are presented in Table 2.

Table 2. Mean, and coefficient of variations by regions and over the entire data

Reg.							DM		NPP				Bim		SYP			
	NPB		PLH		DF						SW					HI		
code*	M	CV	M	CV	M	CV	M	CV	M	CV	M	CV	M	CV	M	CV	M	CV
1	9.4	0.1	113.9	1.9	58.6	0.5	146.5	0.7	434.6	15.8	8.1	0.8	208.8	9.2	94.6	5	45	0.6
2	10	0.2	120.9	2.3	62.1	1.5	153.5	3.2	505.7	26.9	8.3	0.1	259.1	14.9	100.8	5	41	1.0
3	9.3	0.2	102.9	2.3	52.8	0.6	140.7	1.3	363.6	19.8	8.1	0.1	162.4	9.0	78.0	5	52	4.5
4	9.4	0.1	114.5	1.8	49.6	0.5	140.3	0.9	357.4	14.8	9.1	0.3	176.3	7.5	87.6	4	50	1.6
5	8.8	0.1	105.2	2.0	45.3	0.5	136.3	1.0	317.3	15.1	9.1	0.1	148.4	7.3	73.3	4	48	0.8
6	9	0.3	94.1	3.7	44.0	0.9	132.3	1.7	319.3	25.3	7.8	0.2	121.6	10.7	65.9	6	53	1.6
7	9.6	0.4	108.9	6.8	51.0	1.3	147.7	1.8	470.5	53.7	8.9	0.3	230.3	28.7	107.3	13.	44	2.6
Ethiop	9.3	23	110.7	27.3	53.1	17.81	142.8	14.2	392.3	65.6	8.5	30.9	188.1	73.2	86.7	80	47	56

*** 1 = Gojam, 2= Gondar, 3 = Shoa, 4 = Wollo, 5 = Tigray, 6 = Wollega, 7 = Ertria (independent country since 1991)** NPB = Number of primary branches /plant, PLH = plant height, DF = days to flowering, DM = days to maturity, NPP = number of pods /plant, SW = 100 seed weight, Bim = biomass, SYP = seed yield /plant, Hi = harvest index.

Table 3. Mean, and coeffiecent of variations by altitude groups and over the entire data

Reg. code	NPB		PLH		DF		DM		NP	SW			Bim		SYP		HI	
	M	CV	M	CV	M	CV	M	CV	M	CV	M	CV	M	CV	M	CV	M	CV
1	9.6	0.1	12	1.9	52.3	0.7	143	0.8	417.3	16.8	8.4	0.1	204.6	9.7	94.7	5.1	46.4	0.7
2	9.1	0.1	107	1.6	48.8	0.5	139.2	0.8	330.2	12.9	8.9	0.1	159.8	6.5	74.7	3	47.2	0.9
3	9.4	0.1	11	1.6	56.1	0.5	144.2	0.8	415.8	14.1	8.2	0.1	198.6	7.5	89.3	3.2	47.2	1.9
4	9.4	0.2	115	2.2	57.1	1.3	147.7	3.3	429.1	22.1	8.7	0.1	195.5	10.3	92.9	6.2	47.5	2.2
Ethiop	9.3	23.4	111	27.3	53.1	17.8	142.8	14.2	392.3	65.6	8.5	30.9	188.1	73.2	86.7	79	47	55

Table 4. Mean squares for 9 quantitative morphological traits of 50 grasspea populations

Chracter code	Mean sq,for rep	Mean sq,for population	Mean sq. For error	CV	SE
NPB	2.434	2.064**	0.974	10.58	0.14
PLH	128.488	238.661	171.224	11.82	1.85
DF	62.914	171.109**	27.142	9.78	0.73
DM	335.605*	162.003**	73.824	6.01	1.21
NPP	8331.588	30405.988**	11309.8	27.41	15.03
SW	1.19	2.280**	0.949	11.44	0.13
Bim	5557.791	9320.301**	2976.59	29.25	7.71
SYP	746.793	1122.440*	744.363	31.66	3.85
Hi	41.771	183.348**	55.527	15.81	1.05

*,** indicates significance at 5% and 1%, respectively

Table 5. Mean squares for the 50 grasspea populations and 24 individuals with in a population from two-way analysis of variance

VARIABLE CODE

Source	D.F	NPB	PLH	DF	DM	NPP	SW	Bim	SYP	HI
A	49	14.6**	1882.8**	1326.7**	1290.7**	239485.2**	17.8**	72033**	9143**	1431**
I	23	8.4*	1718**	144*	702*	121283*	5.8	27892	6073	1079*
E	1123	4.7	919	89.4	411.7	66428	6.9	90843	6780	687.6

*,** indicates significance at 5% and 1%, respectively

A=populations

I=individuals in a population

E =error

As indicated in Tables 2 & 3, the character that showed the highest coefficient of variation is not the same for all regions and altitude groups. The mean values for each of the 50 grass pea populations in each character is indicated in Appendix 3.

Populations collected from Gondar showed the highest mean primary branches per plant while populations from Tigray showed least primary branches per plant. Plant height showed higher coefficint of variation (6.8%) in populations collected from Eritrea. Populations from Gondar are in general long with mean hieghts of 120.9 cm (Table 2). Total biomass per plant is also high in populations from this region (259.1 g). Populations from Gojam and Gondar regions showed low harvest index while populations from Wollega showed high harvest index.

Variation in phenological traits was observed among populations from different regions. The highest coefficient of variation was observed in Gondar, 1.5% for days to flowering

and 3.2% for days to maturity. Populations from Wollega took minimum days to flower (mean=44) and to mature (mean= 132 days) while populations from Gondar were late both to flower and mature with means of 62 and 154 days, respectively. As indicated in Table 6, days to flower and mature over the entire populations in the country ranged from39-73 and 127-165 days, respectively. Number of pods per plant showed much variation (CV= 53.7%) in Eritrea and this is followed by Gondar (CV= 26.9%). The mean number of pods per plant , however, was highest (506 pods per plant) in Gondar. Seed yield per plant for grasspea was highest from Eritrea (107 g) and this is followed by Gondar (100.8g/plant). The mean seed yield /plant in the country is 86.7 g.

Variations in the various morphological charactes were also observed by altitude groups (Table 3). The highest altitude group (group 4) which includes populations collected from above 2550 m showed higher coefficient of variation for the morphological traits studied. Similarly the mean performance of the populations in this altitude group was much higher than others. As it can be expected days to flowering and days to maturity were minimum for populations in the first two altitud groups. The highest mean seed yield per plant was obtained from low altitude populations which are early maturing indicating the effectiveness of the crop to use efficiently the available short cycle moisture. However, in the lowlands yield of grass pea was reported to be in general low due to aphid damage (Wuletaw, 1997).

Analysis of variance over the entire data showed that highly significant differences between populations were observed for days to maturity, pods per plant, seed yield per

plant, harvest index and dry matter yield (Table 4). Two way analysis of variance was also carried out to determine variation with in a population. Significant difference was observed between individuals in a given population for days to flowering, maturity, number of primary branches per plant, pods per plant and harvest index (Table 5). In general regions and altitude groups with high coefficient of variation are associated with high variability for the particular trait considered.The Gondar region has the highest CV for most traits studied indicating the diversity of each trait in the region.

4.1.2. Heritability (broad sense), Genetic advance and Estimates for Components of Variance

Phenotypic (Pcv) and genetic (Gcv) coefficient of variation, estimate of the component of variance, heritability (broad sense), and genetic advance as percent of the mean were determined (Table 6).

Table 6. Summary statistics and estimates of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) broad sense heritability (H^2) and genetic advance (GS) in 50 grasspea populations for 9 quantitative traits

characters	mean	Range	Vp	Ve	Vg	PCV%)	GCV%	H^2	GS(% of mean)
NPB	9.02	8-12	0.68	0.32	0.36	9.19	6.68	52.9	9.7
PLH	110.7	93-125	79.5	57	22.4	8.05	4.28	28.2	4.6
DF	53.2	39-73	57	9	47.9	14.22	13.02	84.14	24.5
DM	143	127-165	54	25	29	5.13	3.79	54.4	5.7
NPP	387.9	199-675	10135	3769.9	6365	25.9	20.5	62.8	33.5
SW	8.5	7-12	0.76	0.31	0.45	10.2	7.8	59.2	12
Bim	186.5	86-374	3106.8	992	2115	29.8	24.6	68	41.8
SYP	86.2	42-133	374	248	126	22.4	13	33.6	15.2
Hi	47.1	30-85	61	18.5	42.6	16.59	9.1	69.7	23.5

* Vp = phenotypic variance, Ve = error variance, Vg = genotypic variance

Phenotypic coefficient of variation (PCV) was slightly higher than genotypic coefficient of varation for all the characters studied signifying that genotypic factors exerted reasonable effect in estimating the variation . The wide difference between PCV(22.4%) and GCV(13.0%) for seed yield per plant indicates the complexity of this trait and the important role of other factors such as environment in influencing yield potential in addition to the the genetic factors. Similar result was obtained by Belay (1997) in durum wheat.

Most of the characters had high heritability estimates indicating the lesser influence of the environment on them. Days to flowering showed high heritability estimate (h^2= 84.14%). Heritability estimate was low (h^2=33.0%) for seed yield per plant and plant height (h^2=28%) which clearly indicates the major influence of the environment in these characters.

4.1.3. Correlation Analysis of Quantitative Characters

Thorpe (1976) indicated that character associations are important to indicate common elements of epigenetic control and/or similar response of characters to selection pressure. Correlation of the different characters by region and over the entire data in grass pea are indicated in appendix 4 to 8.

Table 7. Correlation among 9 different characters of 50 grass pea populations in Ethiopia

Characte	Bim	DF	DM	HI	NPB	NPP	PLH	SPP	SW	SYP
Bim		0.172**	0.485**	-0.011	0.372**	0.803**	0.576**	0.308**	0.033	0.784**
DF			0.326**	-0.055	0.119**	0.155**	0.09**	0.194**	-0.015	0.086**
DM				0.136**	0.194**	0.518**	0.604**	0.465**	0.044	0.455**
HI					0.004	0.166**	0.111**	0.110**	-0.039	0.207**
NPB						0.397**	0.232**	0.166**	0.017	0.352**
NPP							0.589**	0.379**	0	0.787**
PLH								0.379**	0.005	0.557**
SPP									-0.007	0.298**
SW										-0.003
SYP										

In all the regions(appendix 4-8) and the entire data (Table 7), seed yield per plant was highly correlated with number of primary branches per plant, number of pods per plant and plant height. Similarly there was highly significant correlation between days to flowering and days to maturity. The presence of persistent correlation among most characters considered could be due to natural selection between individuals with in a population which changes the genetic constitution of the population, but the mean fitness will not change if the population is already at the limit of the carrying capacity of its environment which of course is common in most cultivated plants (Endashaw Bekele, 1996).The highly significant correlations between the different characters also indicate

the presence of some common elements of genetic control such as pleiotropy and high linkage between genes.

The effect of environment on the genotypic performance of most traits was explained by many authors (Belay , 1997; Wuletaw Tadesse, 1998). Accordingly splitting of genotypic and phenotypic correlations is important for clear understanding of character associations as indicated in Table 8.

Table 8. Genotypic (G) and phenotypic (p) correlations in 9 quantitative morphological characters of 50 grasspea populations

Characters	NPB	PLH	DF	DM	NPP	SW	Bim	SYP	Hi
NPB (G)		.069	0.625**	0.707**	0.986**	-0.301	0.884**	0.905**	-0.28
(P)		0.273	0.249	0.264	0.634**	-0.11	0.578**	0.537**	0.986**
PLH (G)			0.986**	0.819**	0.606**	0.41**	0.86**	0.676**	-0.677**
(P)			0.261	0.547**	0.606**	0.11	0.605**	0.629**	-0.105
DF (G)				1	0.891**	-.609**	0.956**	1	-1.56
(P)				0.578**	0.407**	-0.314	0.426**	0.205	-0.83**
DM (G)					0.813**	-0.42**	1	0.91**	-0.69**
(P)					0.535**	-0.16	0.55**	0.43**	-0.23
NPP (G)						0.504**	0.991**	0.982**	-0.50**
(P)						0.198	0.85**	0.781**	-0.108
SW (G)							-0.39*	-0.134	0.184
(P)							-0.103	-0.02	0.232
Bim (G)								0.791**	-0.619**
(P)								0.709	-0.285
SYP (G)									-0.606**
(P)									-0.006
Hi									

*, ** indicates significance at 5% and 1%, respectively

Genotypic and phenotypic correlations showed similar signs for most traits except for the association between number of primary branches per plant and harvest index for which the phenotypic correlation is strongly positive while the genetic coefficient is negative. In some cases the phenotypic and genotypic correlations were close in magnitude indicating the less impact of the environments over the traits mentioned. However, in other cases, the magnitude between the two coefficients is high indicating the effect of environmental variances and covariances as indicated in the case of the association between yield and harvest index where the genotypic correlation coefficient and the phenotypic correlation coefficients are -0.606 and -0.006, respectively.

Strong positive genotypic and phenotypic correlations was observed between days to flowering and seed yield per plant. Very strong genetic correlation was also achieved between day to flowering and days to maturity. Similar result was reported by Belay (1997) in durum wheat. Plant height showed strong positive genetic correlation with most of the traits studied except with harvest index. The strong positive association of pods per plant, 100 seed weight and primary branches per plant with seed yield indicates the possibility of selecting lines for yield improvement based on these characters at the very early stage of the breeding program.

4.1.4. Cluster Analysis

Based on the mean agronomic values of both qualitative and quantitative characters, the 50 grass pea populations were grouped in to five clusters (Table 9).

Cluster I consisted three populations which were collected from Tigray. Two of the populations in this cluster are from the same altitude group (1901-2250 m.a.s.l) while the third population is from altitude group 1 (< 1900 m.a.s.l).

Cluster 2 consisted the maximum number of populations (21) collected from Shewa, Wollo, Gojam, Wollega and Tigray. In this group populations from Gondar are not included.

Cluster 3: thirteen populations collected from the different regions including Eritrea are included in this cluster.

Cluster 4: this cluster consisted eleven populations collected from different regions mainly from Gondar.

Cluster 5: Two populations from Gondar were distinctly grouped in this cluster. The two populations in this cluster, however, are different in altitude groups even though they are in the same region. Population 48 was collected from Woreta, a lowland area (1800 m.a.s.l) while population number 49 was collected from Dabat, a very highland area (2730 m.a.s.l). This result clearly indicates the lesser role of altitude for genetic diversity. Similar result was obtained by clustering the 50 grass pea populations based on quantitative characters only. On the other hand, clustering based on qualitative characters (flower color, seed color, pattern of testa) resulted in to 4 clusters (Table10). In this case much of the populations (70%) were grouped in cluster 1. Cluster 4 consisted only one population which was collected from Tigray.

Table 9. Average linkage clustering of 50 grasspea populations based on qualitative and quantitative morphological characters

Cluster	Accession code
I	24, 27, 32
II	8, 28, 2, 35, 5, 12, 19, 22, 40, 1, 4, 9, 44, 41, 25, 20, 39, 11, 21, 30, 47
III	3, 37, 7, 33, 15, 31, 43, 13, 23, 16, 17, 45,46
IV	10, 26, 18, 38, 14, 34, 36, 29, 42, 50, 6
V	48, 49

Table 10. Average linkage clustering of 50 grasspea populations based on qualitative morphological characters

Cluster	Accession code
I	4, 24, 14, 47, 11, 50, 16, 18, 21, 41, 44, 1, 40, 6, 7, 42, 10, 35, 49, 34, 37, 8, 3, 28, 15, 39, 38, 48, 43, 45, 20, 13, 2, 36, 25, 17, 12
II	85, 31, 46, 19, 29, 23, 22, 26
III	9, 27, 30, 32
IV	33

In general, the variation based on qualitative traits is much less among populations of the different regions. Similar result was obtained by Jackson & Yunus (1984). Clustering of the populations based on quantitative morphological characters and biochemical results

(ODAP and protein) content is indicated in dendrogram (Fig. 2). Three major clusters C I, C II, & C III are resulted. Cluster I (CI) contains 45 populations. It is however, sub-divided in to different sub-clsters indicating the great intraclass variation. Cluster II (C II) contains only one population (population no. 43) indicating that this population is different from all the other populations. This population was collected from Gojam region, Bahir Dar district at 1685 m. a. s. l. (which is low land for grasspea production). This population was grouped in Cluster I together with most of the populations when qualitative morphological characters were used as classifying variables (Table 10)

Cluster III (CIII) contains only four populations , population no.s 24, 27, 32 (from Tigray) and population no. 47 from Gojam. The overall clustering result indicates the lack of consistency in classification of the populations when different classifying variables are considered.

Hierarchical cluster analysis was performed using the region of origins centroids of the six significant canonical functions. The dendrogram from this analysis (fig 3) reveals that the Gojam and Gondar regions are in the same cluster while Shoa and Wollo belong in the same group. The Tigray and Wollega region are grouped in the same cluster. The grouping of these two geographically distant regions maye be due to migration of people from the Tigray region to Wollega region. But this needs further study since the number of populations used for this study from Wollega region are few in number.

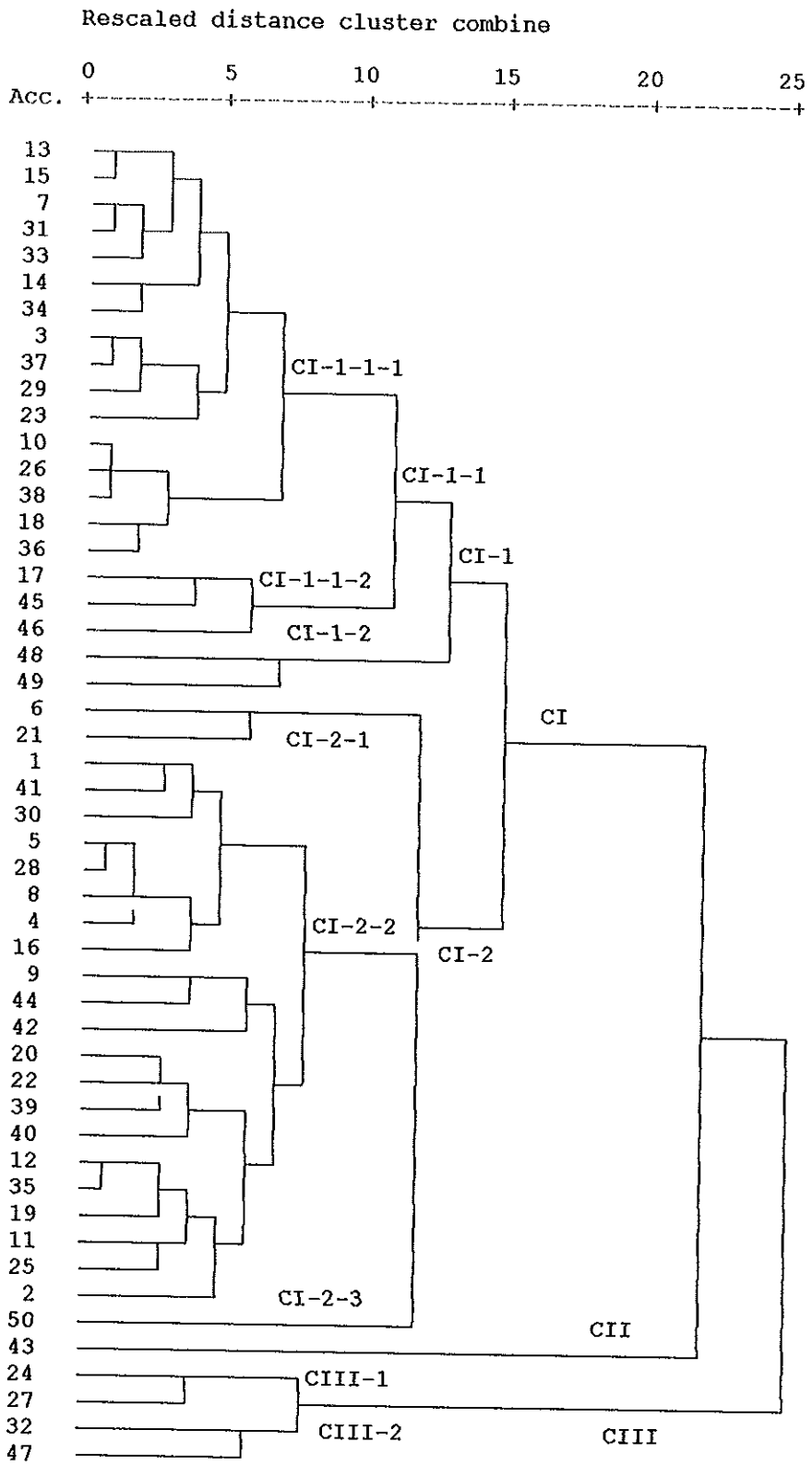


Fig 2. Dendrogram using Average Linkage on 50 grasspea populations based on Quantitative morphological characters, protien and ODAP contents.

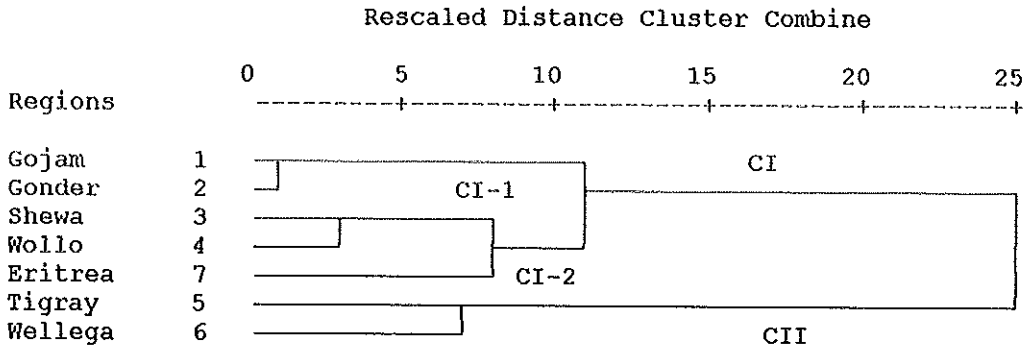


Fig 3. Dendrogram using Average Linkage (Between Groups)based on regions from hierarchial clustering of six significant canonical functions.

4.1.5. Factor Analysis

Factor analysis is a type of multivariate analysis that can be used to reduce a large number of correlated variables to a smaller number of main factors, thus helping to elucidate the number and nature of causative influences and aiding in the selection of better genotypes (Seiler and Stafford, 1985).

Table 11. Eigen values for different factors on 10 different characters of 1199 grasspea entries

Character	Factor	Eigen value	Pct var.	Comm %
Df	1	3.97	39.7	39.7
Bim	2	1.14	11.5	51.2
Dm	3	1.09	10.9	62.1
Hi	4	0.99	9.9	72.1
NPB	5	0.82	8.3	80.3
NPP	6	0.73	7.3	87.7
Pht	7	0.52	5.3	93
Spp	8	0.33	3.3	96.3
Sw	9	0.2	2.1	98.3
SYP	10	0.1	1.7	100.0

The factor corresponding to the largest eigen value is the factor that accounts for greatest amount of variation. Accordingly factor 1 with eigen value of 3.97 accounts for about 40% of the variation. A principal factor matrix after varimax rotation for the 10 variables is given in Table 12.

Table12. Principal factor matrix after verimax rotation for 10 characters of 1199 individual of grasspea

Variables	factor 1	factor 2	factor 3	communality
Df	-0.039	0.776	-0.248	0.666
Bim	0.878	0.187	-0.122	0.82
Dm	0.484	0.667	0.149	0.701
Hi	0.142	-0.038	0.826	0.705
NPB	0.55	0.009	-0.294	0.389
NPP	0.89	0.172	0.054	0.825
Pht	0.675	0.343	0.166	0.601
Spp	0.263	0.642	0.227	0.534
Swt	0.076	-0.039	-0.39	0.159
Syp	0.886	0.088	0.119	0.807

Factor analysis was carried out for 10 morphological characterstics and the eigen values for each factor is indicated in Table 11.The factor analysis technique divided the 10 variables into three groups or factors. For purposes of interpretations only those factor loadings greater than 0.5 were considered important. A summary of the composition of variables of the three factors with loadings is given in Table 13. The three factors account for about 62% of the total variation (Table 11).

Table 13. Summary of factor loadings for 10 characters measured on 1199 indivisuals of grasspea

Factor 1	**Loading**	**Suggested factor name**
Bim	0 878	**Productivity factor**
NpB	0.55	
NPP	0.89	
PLH	0.675	
SYP	0.886	
Factor 2		
DF	0.776	
DM	0.667	**Reproductive factor**
DM	0.667	
Spp.	0.642	
Factor 3		
HI	0.826	**Efficiency factor**

Factor 1, which accounted for about 40% of the variation was strongly associated with biomass(Bim), number of primary branches (NPB), number of pods per plant (NPP), plant height (PLH) and seed yield per plant (SPP). This factor was regarded as productivity per plant factor since it included several traits which are components of yield. Factor 2 which accounts for about 12% of the variation was named as reproductive (fertility) factor since it consisted days to flowering (DF), days to maturity (DM) and

seeds per pod (SPP) which are associated with fertility. The third factor was named efficiency factor since it contains harvest index (HI) which is the ratio of yield to the total biomass, which will measure the efficiency of the plant to convert the available resource into seed yield. The present study is similar with the diversity study reported by Rao and Paroda (1982) in cluster bean using factor analysis.

4.1.6 Frequeny Distribution and Diversity Index for some Selected Morphological Characters

4.1.6.1 Regional Trait Distribution

All regions except Eritrea and Wollega were represented by more than 5 populations. Small leaflet size was predominant in all regions and in the country in general followed by medium leaflet size. In Gojam and Wollo, 80% of the population had small leaflet size while in Gondar and Shoa the percentages for this class were 73 and 75%, respectively. The large leaflet size was rare in all regions with mean frequency of 2% in the country.

Blue flower color was expressed dominantly in each region and the country as a whole. In Gojam region, population 46012, 46042 and 236686 were monomorphic for blue flower color. Similarly, population 46020 in Shoa region, population 226001 in Wollo region and population 207497 in Tigray region were also monomorphic for flower color since all showed blue flower color. Regionally, maximum variation for this trait was observed in Gondar. The common occurence of blue flower color in particular and the less variability of grasspea for this trait was also reported by Jackson & Yunus (1984).

Most populations in each region showed medium seed size while large seed size percentage distribution was the least both in regions and the country in general. The frequency of each trait in each region is indicated in appendix 9.

Variations in seed colour and size with in a population is indicated in fig.4. Out of the four types of seed colors, olive was dominantly distributed in all regions and the country with mean national percentage frequency of 50%. But black seed color is rare with mean national percentage frequency of 4%. The pattern of testa in most populations was spotted type with percentage frequency of 52, 63, 64 and 65% for Gojam, Gondar, Shoa and Tigray regions, respectively.However all types of patterns of testa were observed in each region. There was highly significant difference in each regions for each trait as indicated in the chisquare analysis (Table 14)

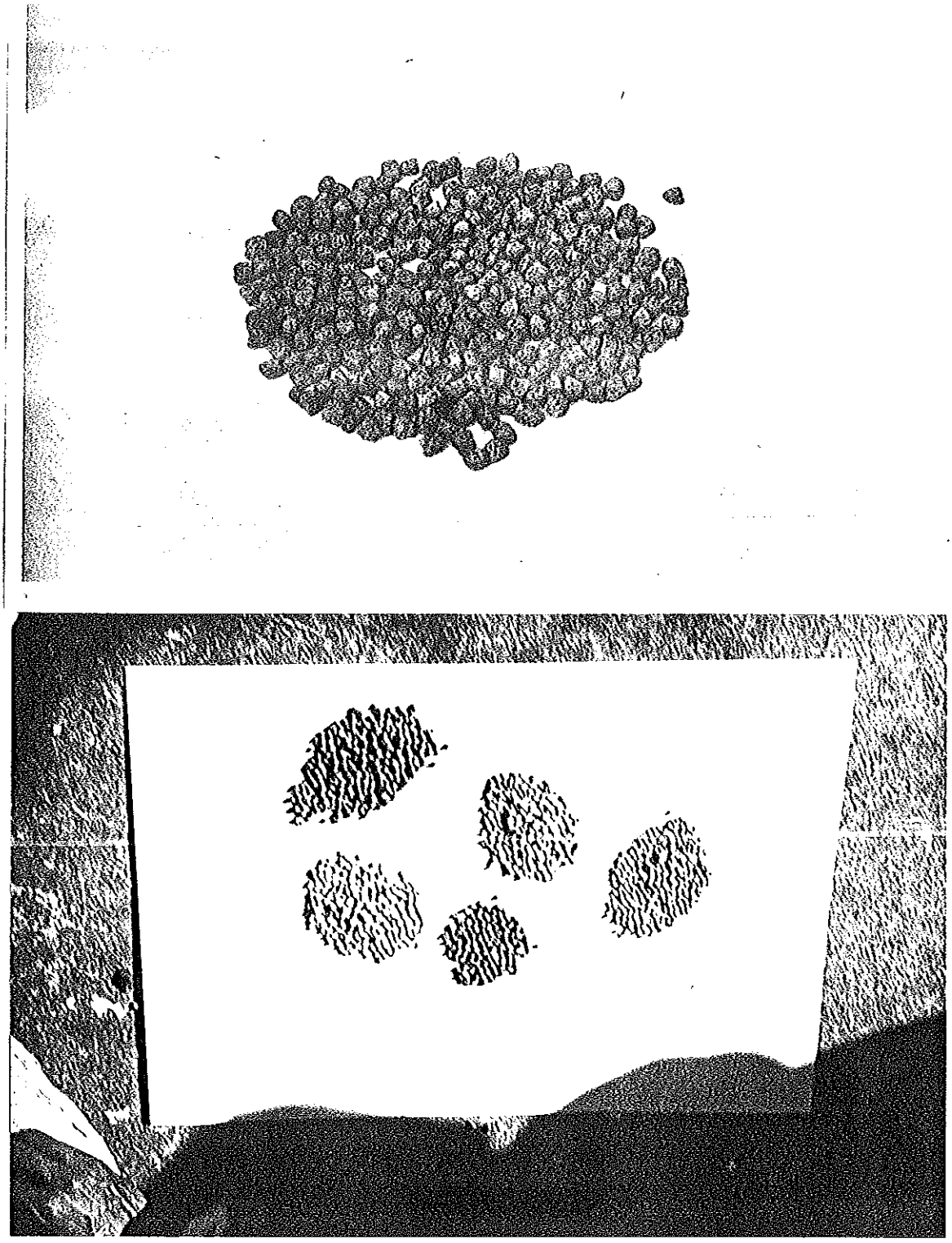


Fig. 4 Variation in seed colour and seed size with in a population.

Table 14. Chisquare value for five administrative regions for five morphological characters

Region	Flower color	Leaflet size	Seed color	Pattern of testa	Seed size
Gondar	116.6**	131.1**	141.1**	116.7**	55.1**
Gojam	655*	327.9**	209.9**	238**	333.2**
Shoa	3067**	1167.2**	1088.2**	871.5**	1931.8**
Tigray	177.1**	267.1**	302.1**	173.7**	161.2**
Wollo	413.1**	211.5**	254.5**	219.8**	173**

** indicates significance at 1%

4.1.6.2 Altitudinal trait Distribution

The phenotypic frequencies for individual characters and altitude classes as percentages of the number of genotypes from each altitude class by pooling populations together is summerized in table 15. The third altitude class contains the maximum number of entries (384) where as the fourth class (the higher altitude class) contains the least number of entries (167).

Table 15. Percentage of phenotypic classes in each trait and altitude group.

altitude*	NO	S	M	L	B	P	R	A	B	C	Og	G	Br	Bl	1	2	3	4
1	288	77	22	1	77	22	1	19	73	8	52	5	40	3	11	63	18	8
2	360	79	20	1	80	20	0	16	63	21	45	8	44	3	8	66	20	6
3	384	82	16	2	87	12	1	25	67	8	52	3	39	6	10	58	25	7
4	167	78	20	2	89	11	0	26	66	8	52	5	40	3	10	60	22	8

* 1 = < 1900 m, 2 = 1901-2250; 3 = 2251-2550 m; 4 = >2551 m

There is no much variation in leaflet size, seed size and seed color by altitude groups. However, the distribution of pink flower color is high in the lower altitude classes than

the higher altitude classes. In general the variation of the traits conciderd were less variable by altitude groups than by regions.

4.1.6.3 Diversity Index

Estimates of diversity for individual characters, populations, regions and altitude classes are shown in table 16 and Fig 5, respectively. Polymorphism was common in varying degrees for most characters indicating the existence of variation in the populations studied. H' values for flower color in Gojam ranged from 0 (monomorphic) to 0.57 (medium diversity). The highest mean diversity index (H') pooled over traits was shown by populations from Gondar (H'=0.65) followed by Tigray (H'= 0.64). There is no significant variation for mean diversity index between regions. Significant variation, however, was observed with in regions (Table 17).

Table 16. Diversity index for each population in each region.

Region	leaflet size	flower color	seed size	seed color	pattern of testa	mean±SE
Gojam						
46012	0.46	0	0.7	0.56	0.9	0.52
46030	0.14	0.61	0.3	0.79	0.85	0.53
46042	0.49	0	0.73	0.68	0.65	0.51
46044	0.24	0.14	0.73	0.51	0.66	0.45
46049	0.6	0.57	0.82	0.58	0.49	0.61
46050	0.46	0.49	0.59	0.59	0.69	0.56
46073	0.4	0.13	0.49	0.75	0.75	0.5
208449	0.77	0.4	0.6	0.49	0.77	0.6
226019	0.68	0.32	0.6	0.64	0.73	0.59
228495	0.34	0.24	0.88	0.59	0.64	0.53
236711	0.57	0.3	0.71	0.75	0.77	0.62
236686	0.24	0	0.7	0.54	0.65	0.4
236701	0.46	0.57	0.5	0.45	0.55	0.5
Region	0.5	0.34	0.71	0.67	0.81	0.60+0.1
Gondar						
46033	0.55	0.34	0.82	0.62	0.81	0.62
207493	0.77	0.3	0.57	0.74	0.64	0.60
207499	0.81	0.4	0.6	0.69	0.8	0.66
226014	0.32	0.34	0.7	0.75	0.77	0.57
46107	0.54	0.14	0.59	0.69	0.83	0.63
236705	0.56	0.54	0.5	0.44	0.43	0.49
236708	0.14	0.14	0.75	0.64	0.59	0.42
Region	0.62	0.36	0.8	0.71	0.75	0.65+.09
Shoa						
46003	0.6	0.46	0.73	0.59	0.43	0.56
46008	0.77	0.24	0.6	0.75	0.66	0.60
46016	0.47	0.27	0.34	0.69	0.88	0.53
46020	0.57	0	0.4	0.64	0.58	0.43

46023	0.55	0.24	0.82	0.59	0.83	0.61
46024	0.54	0.45	0.64	0.69	0.64	0.59
46027	0.32	0.24	0.49	0.69	0.75	0.49
Region	0.57	0.29	0.61	0.63	0.73	0.56+.1
Wollo						
46034	0.46	0.57	0.51	0.58	0.67	0.55
46035	0.32	0.7	0.7	0.39	0.65	0.55
46099	0.32	0.54	0.64	0.75	0.79	0.61
46100	0.32	0.32	0.64	0.46	0.46	0.44
46106	0.46	0.75	0.77	0.59	0.51	0.61
46111	0.61	0.32	0.64	0.48	0.62	0.53
215247	0.57	0.14	0.49	0.58	0.31	0.42
226001	0.32	0	0.61	0.55	0.69	0.43
22606	0.32	0.55	0.77	0.64	0.64	0.58
226010	0.55	0.75	0.49	0.59	0.58	0.59
Region	0.45	0.52	0.66	0.62	0.67	0.58+.02
Wollega						
46110	0.41	0.7	0.79	0.74	0.74	0.67
211511	0	0.62	0.61	0.65	0.56	0.48
Region	0.24	0.55	0.73	0.72	0.66	0.58+.1
Tigray						
207496	0.46	0.32	0.4	0.69	0.72	0.52
207497	0.14	0	0.14	0.54	0.74	0.31
207566	0.14	0.5	0.59	0.58	0.37	0.43
207567	0.4	0.32	0.64	0.84	0.65	0.57
219945	0.4	0.54	0.68	0.69	0.64	0.59
219946	0.61	0.6	0.55	0.45	0.23	0.48
219949	0.4	0.57	0.74	0.49	0.54	0.54
219950	0.46	0.4	0.73	0.64	0.77	0.60
219952	0.46	0.5	0.68	0.75	0.31	0.54
223219	0.4	0.32	0.46	0.49	0.19	0.32
Region	0.42	0.52	0.85	0.75	0.68	0.64+.09
Eritrea						
220118	0.4	0.5	0.66	0.58	0.58	0.54+.04
Ethiopia	0.52	0.41	0.79	0.71	0.62	0.61

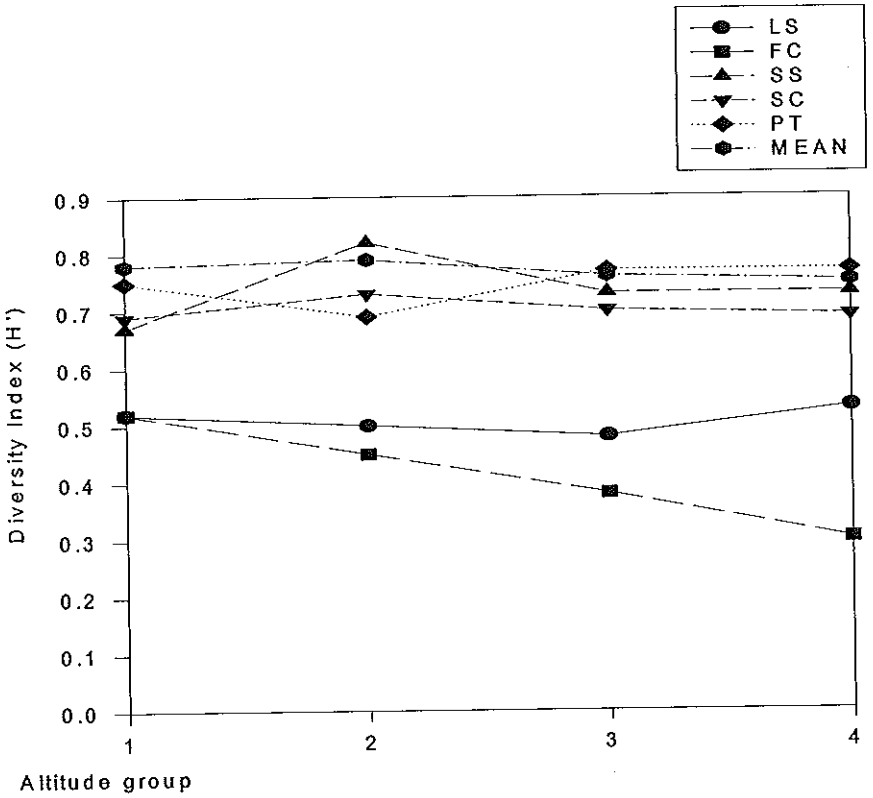


Fig 5. Mean diversity indices (H') for each of five traits and the over all mean across traits in four altitude groups.
Ls: leaflet size Fc: flower color Ss: seed size Sc: seed color
Pt: patern of testa

Table 17 Analysis of variance for Shanon Weaver Diversity index between regions

Source	Deg of fred.	Sum of squares	mean squares
Between groups	6	0.0713	0.119
with in groups	28	0.986	0.0352*
Total	34	1.0582	

* indicates significance at 5%

4.2. Biochemical Diversity

4.2.1 Oxalyl Di-amino propanoic acid (ODAP) and protein variation.

Analysis of variance (Table 18) showed that there is no significant variation among populations for both ODAP and protein contents. ODAP analysis from single plants showed significant variation with in a population(Table 19). The mean ODAP and protien contents of the 50 grasspea populations are indicated in Appendix 10 and the ODAP value for 150 individual plants selected from the 50 populations is indicated in Appendix 11. The variaion of ODAP between single plants ranged from 0.14 to 0.91%. Fortunately, out of the 150 individual plants from the 50 grasspea population used in this study, 4 individual plants were in the safe range (below 0.2%). The first individual with very low ODAP content was from Showa region (46027-r2-3) with ODAP value of 0.14%. Three other individuals were from Wollo, Gonder and Tigray (46106-r2-3, 46033-r2-2 and 207497-r1-5), respectively.

Table 18. Analysis of variance for ODAP and Protein content of grasspea populations using different regions

Source	Deg. fre.	Mean sq for ODAP	Mean sq. for protein
between regions	6	0.0057 (NS)	2.4615 (NS)
Between populations	43	0.0060 (NS)	3.2706 (NS)
Total	49		

Table 19. Analysis of variance for ODAP content of individual plants in the entire data

Source	Deg. fre.	Sum of squares	Mean squares
between	2	0.178	0.089*
with in	147	3.069	0.021*
Total	149		

*, indicates significance at 5%

Correlation of ODAP with protein content and other agronomic characters in two randomely selected regions and over the entire data is indicated in Table 20. There was no significant correlation between ODAP and other characters both in the two regions and the entire data.

The association of protein content with ODAP was significant in Gondar region while positive but non significant in Tigray and over the entire data. This positive association

indicates the difficulty to improve the two traits at the same time. The positive association of protein content with seed yield in the two regions and over the entire data is encourging to improve the two traits simultaneously. ODAP was negatively associated with grain yield both in the two regions and over the entire data (Table 20). Positive association of ODAP with gain yield was reported earlier by Dahiya(1986) and more recently by Wuletaw Tadesse (1998) and Wuletaw *et al* (1997a). On the otherhand, negative association of ODAP with grain yield was reported by (Woldeamlak and Alelign, 1990).

Table 20. Correlation of ODAP content with protein content and other morphological characters in 50 grasspea populations of two selected regions and over the entire data

Reg. Code		OD.	prot.	NPB	PLH	DF	DM	NPP	SW	Bim	SY
2	OD	1	0.81*	0	-0.6	-0.24	-0.3	0.03	-0.29	-0.2	-0.
2	prot	.81*	1	0.3	-0.8*	-0.6	-0.02	0.29	-0.34	0.03	0.6
5	OD	1	0.51	0.2	-0.2	-0.35	-0.34	-0.3	-0.28	-0.3	-0.
5	prot	0.51	1	0.5	0.5	0.51	0.55	0.67*	0.31	0.67*	0.5
Eth	OD	1	0.07	0	-0.2	-0.13	-0.16	-0.2	-0.16	-0.3	-0.
Eth	prot	0.07	1	0	0.07	-0.14	-0.03	-0.1	0.25	0	0.2

In most reports ODAP was positively correlated with seed yield. Quader *et.al.* (1989) observed that lines having low neurotoxine content were of short duration in maturity and low yielding and that high yielding lines had high toxin content. However, Kaul *et.al.* (1986) did not find any consistant asssociation between ODAP content and morphological traits. A wider array of germplasm needs to be studied to establish any such relationship.

4.2.2 Isozyme Study

4.2.2.1 Population variability

The genetic variability at seven loci in all populations is indicated in Table 21. The mean number of alleles per locus ranged from 1.6 to 2.1, the lowest being for population 219950 while the highest being for population 236705. The percentage of polymorphic loci ranged from 57.1 to 85.7. The lowest range was for population 219950 and the highest range was for populations 236705 and 46024. In this case, a locus is said polymorphic if more than one allel was detected. As per this criteria, polymorphism was detected in all populations. The highest polymorphism was detected from populations collected in Gondar region. This is in line with the morphological data. Mean hertrozygosity for the populations ranged from 0.081 in population 46035 to 0.313 in population 226001. The number and type of alleles in each locus for each population is indicated in appendix12. Variations with in and between populations for the different enzyme systems are indicated in figures 6 to 8.

Table 21. Genetic variability at 7 loci in all populations

Population	Mean no. of alleles per locus	Percentage of loci polymorphic	Expected hetrozygosity
A. 208449	1.7	71.4	.189
B . 207499	2.0	71.4	.214
C. 46035	1.9	71.4	.081
D. 219945	1.9	85.7	.302
E. 46024	1.9	85.7	.297
F. 219950	1.6	57.1	.196
G. 226001	1.9	71.4	.313
H. 46027	1.7	71.4	.196
I. 236711	1.7	71.4	.170
J. 236705	2.1	85.7	.241

As indicated in Table 22, marked differences in the extent of differentiation (FST)were shown between many loci. The populations were differentiated among themselves markedly for AAT-1, AAT-2, and EST-2. The level of differentiation was low for ACP-3 (0.031) and for ACP-1 (0.118). The degree of differentiation (FST) of the individual loci ranged from 0.031 for ACP-3 to 0.784 to AAT-2. The mean FST value, 0.346, is medium as compared to the average FST value for inbreeding species (0.510) reported by Hamrick & Godt, 1990.

Table 22. Summary of F-statistics at all loci

Locus	F(ST)	Number of allels
AAT-1	.6302	2
AAT-2	.784	2
EST-1	.188	2
EST-2	.353	4
ACP-1	.118	2
ACP-2	.157	3
ACP-3	.031	3
Mean	.346	

The high FST value reflects adaptation to strong envirnmental disimilarities or high level of genetic drift maintained by restricted gene flow between populations. Similar observations were noted on barley by Abebe and Bjornstand(1997).

The unbiased minimum distance (Table 23) between populations ranged from 0.03 to 0.341. The highest distance (0.341) was between population 219950 from Tigray region and population 46035 from Wollo region.. These two populations were from different regions with much physical distances. This indicates that isolation by and physical distance is one of the important factors responsible for the observed genetic distance disparity.

Table 24. Matrix of genetic distance coefficients

Below diagonal: Nei (1978) unbiased minimum distance

Population	1	2	3	4	5	6	7	8	9	10
1 A	*****									
2 B	.067	*****								
3 C	.117	.174	*****							
4 D	.068	.031	.164	*****						
5 E	.109	.039	.183	.001	*****					
6 F	.212	.152	.341	.151	.155	*****				
7 G	.262	.231	.213	.240	.245	.097	*****			
8 H	.058	.044	.140	.008	.021	.132	228	*****		
9 I	.078	.040	.202	.030	.037	.204	.278	.067	*****	
10 J	.179	.180	.120	.180	.214	.285	.166	.168	.255	*****

Clustering of the populations using unbiased minimum distance was carried out using unweighted pair group method (Fig.9). The populations were grouped in two major clusters. Cluster I contains populations F and G which are collected from Tigray and Wollo regions, respectively. Cluster II which contained 80 % of the populations showed two major sub clusters, 1 & 2. Population 208449 (A) from Gojam and Population 207499 (B) from Gondar were grouped in the same subcluster. This result indicates the similarity of populations collected from nearby adminstrative regions may be due to ecological similarity and /or cultural similarity which inturn results in exchange of grasspea populations in market days and other circumstances.

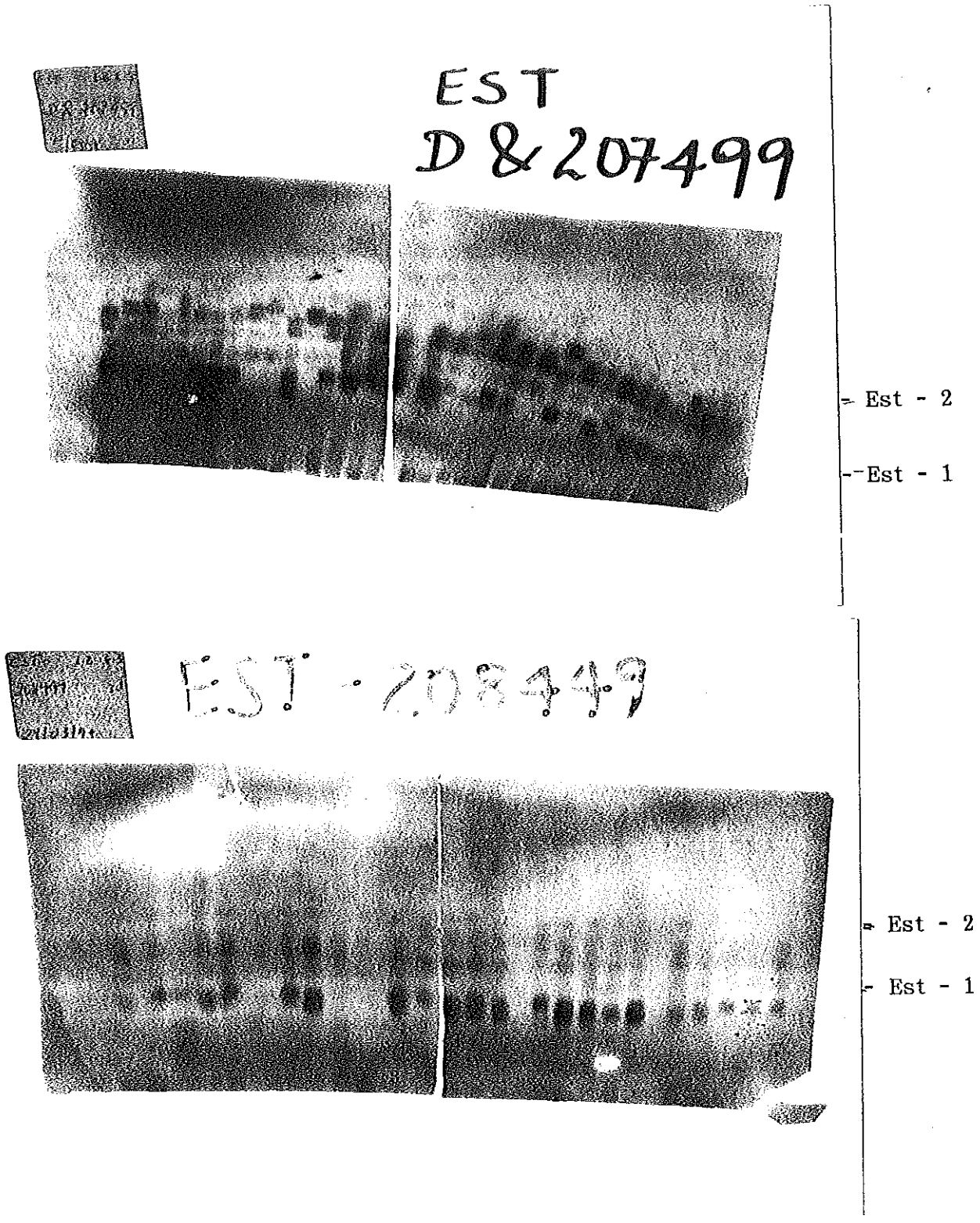


Fig. 6 Variation between and with in populations for esterase (EST)

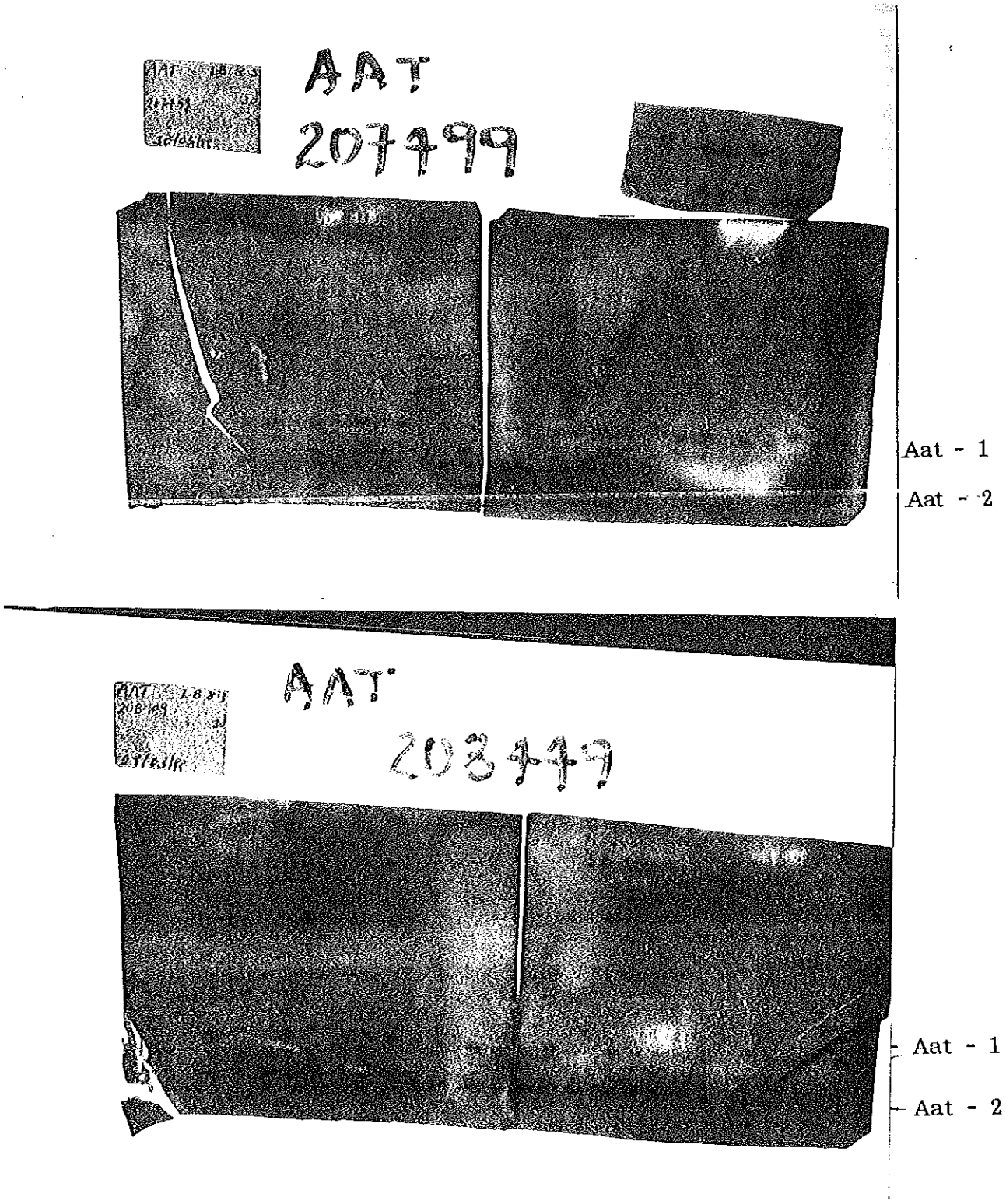


Fig. 7 Variation between and with in populations for AAT

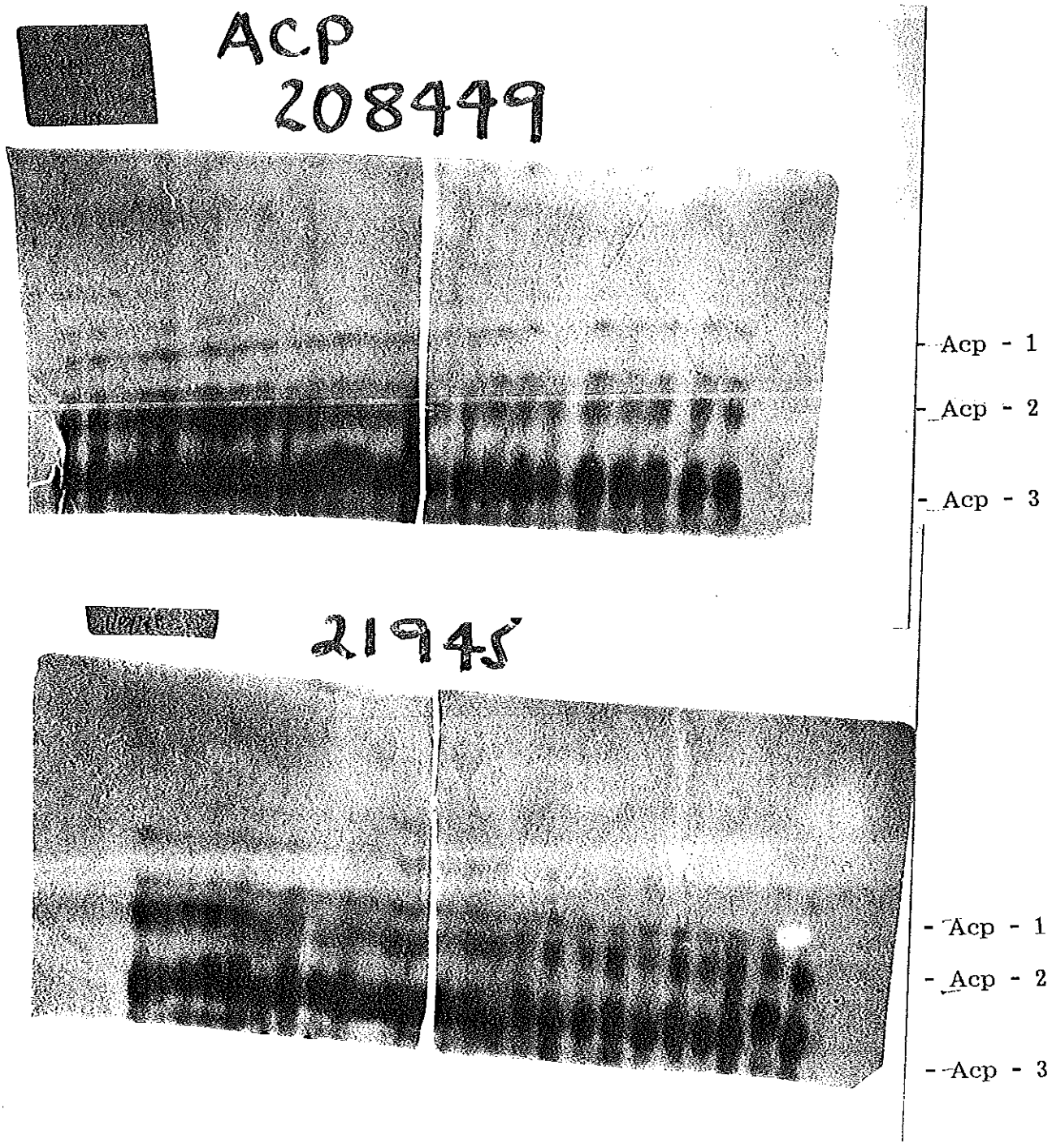


Fig. 8 Variation between and with in populations for ACP.

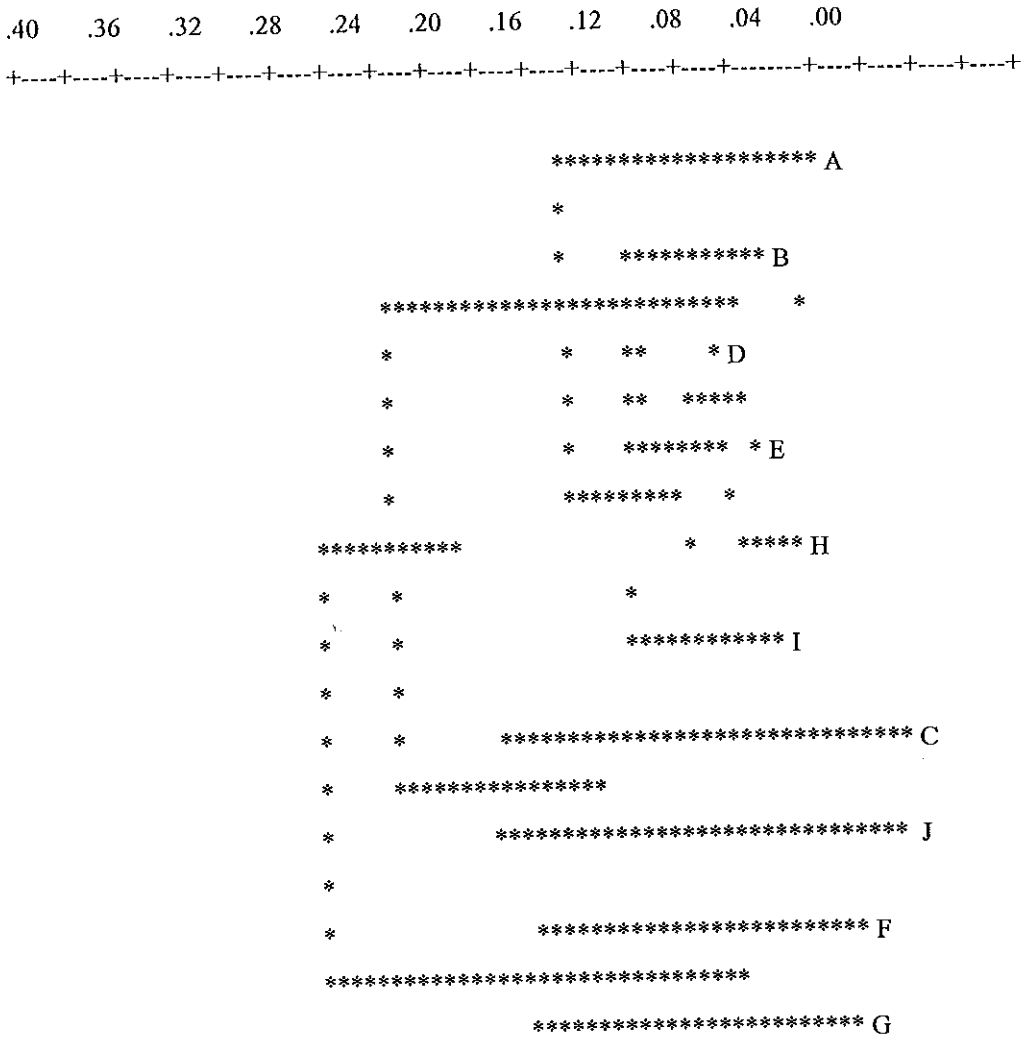


Fig. 9. Dendrogram showing the clustering of the 10 grasspea populations based on Isozyme data

4.2.2.2 Correlation Between Morphologicl and Isozyme Diversity Indices

The association between morphological diversity estimates (Shanon weaver diversity index) and genetic diversity estimates from isozyme data (expected hetrozygosity estimate, H) at population level was negative but nonsignificant (r=-0.25). Yunus *et. al.* 1991 also observed the absence of correlation of isozyme data with morphological data in grasspea. The absence of correlation between markers indicates that there is no one best marker that can be used for diversity study. Hence, it is important to study diversity by using both morphological and molecular markers. Similar result was obtained by many authors for other crops (Seifu, 1997; Abebe & Bjornstad, 1997; Price *et .al.*1984).

5. SUMMMARY AND CONCLUSION

Lathyrus sativus (grasspea) is a popular food and feed crop in countries such as India, Pakistan, Bangladesh and Ethiopia because of its resistance to drought, flood and moderate salinity. Unlike other legumes, it thrives very well under adverse climatic conditions, and requires very little if any, management inputs and attention during its growth cycle when other crops fail under adverse climatic conditions. Grasspea become the only available source of food for the poor section of the population and sometimes a survival food especially in time of drought-induced famine. Although seeds of grasspea are tasty and protein rich (30%), excessive consumption of the seeds causes an upper motor neurone disease called neurolathyrism which is characterized by the paralysis of the lower limbs. The neurotoxic causal agent of this disease is believed to be a non protein aminoacid, Oxalyl Di aminopropanoic acid, (ODAP).

Morphological marker analyses and isozyme analyses has been used widely to estimate genetic variability of crop populations. These methods have been useful in addressing questions on population genetic structure and genetic conservation. Knowledge of genetic diversity of species is particularly important, since modern breeding practices have narrowed the genetic diversity of cultivated crops. In the case of grasspea, the crippling disorder (lathyrism), associated with heavy consumption of the grain, is a discouraging factor for its production. This problem associated with the cereal based extension program leads to banning of grasspea production which inturn aggravates genetic erosion and loss of diversity of the crop.

This reduction in genetic diversity could severely limit future improvement works for low/ zero ODAP content and for adaptive traits such as resistance to biotic and abiotic stresses and reduce stability of crop yields.

The present study was undertaken to investigate the morphological and biochemical variation of grasspea in Ethiopia in order to identify sites for *in-situ* conservation and to aid future germplasm collection.

The morphological diversity study showed that the highest polymorphism is concentrated in Gondar region (H' =0.65) followed by the Tigray region (H' = 0.64). This negates partly the earlier report by Melaku (1988) which was based on the preliminary field survey.

The analysis of variance for H' showed no significant variation between regions which can be explained interms of the environmental hetrogenity of regions, irrelevance of adminstrative and regional boundaries in maintaining diversity, and the significant degree of seed migration along regions. Similarly the diversity indices between the different altitude groups is not significant indicating the less pronounced role of altitude for maintaining diversity.

Allelic polymorphism was detected for all populations at all loci. However, AAT-2, AAT-1 and EST-2 showed the highest polymorphism. Populations 207499 and 236705 from Gondar showed high polymorphism for esterase. These populations were also the

highest in morphological diversity (H') indicating positive association between the two markers. However, such correlation analyses between morphological and biochemical diversity based on all 10 populations was negative (r = -0.25) though non significant.

The ODAP analyses from single plants enabled in the identification of lines with very low ODAP content (0.14%) indicating the potential variability among landraces to screen for low ODAP content and there by to tackle lathyrism which of course, will lead grasspea from a threat to promise. These results will enable to use these low ODAP lines for future breeding program so as to exploit the immense potentials of this hardy pulse and there by utilize it widely for sustainable agricultural development especially in the drought prone areas of the country. However, stability study is important since ODAP is highly influenced by environment (Wuletaw Tadesse 1998; Dixit *et. al.*, 1997; Khawaja, 1997).

The present study also suggests that to tackle the genetic erosion, *in-situ* conservation sites should be primarily selected in Gondar region (representing for Gondar and Gojam) and site from Tigray region followed by sites from Shoa which can represent the Wollo, and Shoa regions since these regions are in the same sub-cluster as indicated in fig 7. Priorities for germplasm collection should also focus in regions of much diversity with due consideration to the cause of genetic erosion and environmental degradations. Further study should also focus on the identification and measuring diversity of wild relatives using the possible available markers in order to improve the crop and tackle neurolathyrism through conventional breeding and biotechnological techniques.

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Appendix 1. Plant species that are believed to be indegeneous to Ethiopia, cultivated crop species that have wide diversity and some of their wild and weedy relatives

Scientific name	Common name	Use
1. *Abelmoschus esculentus*	okra	vegetable
2. *Aframomum korarima*	korerima, cardamon	spice
3. *Amorphophallus abyssinica*	"bagana"	root crop
4. *Allium cepa*	onion	vegetable
5. *Allium sativum*	garlic	vegetable
6. *Avena abyssinca*	oat	cereal
7. *Avena barbarata*	wild oat	weed/wild
8. *Avena waviloviana*		weed
9. *Brassica carinata*	abyssinian mustard	oil crop
10 *Brassica nigra*	black mustard	spice
11. *Brassica campestries*	rape	oil crop
12. *Brassica juncea*	indian mustard	spice
13. *Capsicum species*	chili pepper	spice
14. *Carthamus tinctorius*	saf flower	oil crop
15. *Carthamus flavescens*		wild
16. *Carum copticum*	"nech azmud"	spice
17. *Catha edulis*	Chat	stimulant
18. *Cicer arietinum*	chickpea	pulse crop
19. *Cicer cuneatum*		wild

Appendix table 1 continued

Scientific name	Common name	Use
20. *Coccina abyssinica*	"anchote"	tuber crop
21. *Coffea arabica*	coffee	beverage
22. *Coleus edulis*	'oromo dinich"	tuber crop
23. *Coriandrum sativum*	corriander	spice
24. *Crambe abyssinica*		oil crop
25. *Crambe hispanica*		oil crop
26. *Discorea abyssinica*	yam	root crop
27. *Discorea bulbifera*	potato yam	root crop
28. *Discorea dumentorum*	bitter yam	root crop
29. *Discorea sansibarensis*		wild
30. *Discorea schimpperi*		wild
31. *Dolichos lablab*	hyacinth bean	pulse crop
32. *Elucine africana*		weed/wild
33. *Elucine coracana*	finger millet	cereal
34. *Elucine indica*	fowl foot grass	weed/fiber crop
35. *Elucine tocussa*		cereal
36. *Embelia schimperi*	"enkoko"	medicinal
37. *Ensete ventricosum*	enset	root & tuber crop
38. *Eragrostis tef*	teff	cereal
39. *Gossypium herbaceum*	cotton	fiber crop

Appendix table 1 continued

Scientific name	Common name	Use
40. *Gossypium acerifolium*	cotton	fiber crop
41. *Guizotia abyssinica*	noug	oil crop
42. *Guizotia scabra*		wild
43. *Hagenia abyssinica*	"kosso"	medicinal
44. *Hordeum vulgare*	barley	cereal
45. *Hordeum irregulare*	barley	cereal
46. *Hordeum spontaneum*	barley	wild
47. *Hebiscus canabinus*	kenaf	fiber crop/pot herb
48. *Indigoffera arrecta*	indigo	dye
49. *Indigofera argentea*	indigo	wild
50. *Lagenaria abyssinica*	bottle gourd	container
51. *Largenaria sceraria*	bottle gourd	container
52. Lathyrus sativus	grasspea	pulsecrop
53. *Lens esculenta*	lentil	pulse crop
54. *Lepidium sativum*	cress, "feto"	medicinal
55. *Linum usitatissium*	linseed	oil crop
56. *Lupinus albus*	lupin	pulse crop
57. *Nigella sativa*	black cumin	spice
58. *Pennisetum typhoides*	bulrush millet	cereal
59. *Pisum sativum/abyssinicum*	fieldpea	pulsecrop

Appendix table 1 continued

Scientific name	Common name	Use
60. *Rhamnus prinoides*	"gesho"	used as hops
61. *Racinus communis*	castor bean	oil crop
62. *Ruta gaveolns*	rue	spice
63. *Sesamum indicum*	sesame	spice
64. *Sorghm bicolor*	sorghum	cereal
65. *Triticum durum*	wheat	cereal
66. *Trigonella foenum-graecum*	fenugreek	spice
67. *Vicia faba*	fababean	pulse crop
68. *Vigna unguiculata*	cowpea	pulse cop

Source: PGRC/ILCA Newsletter No.1, December 1982

Appendix 2. Physiographic and soil characteristics of the test location, Adet

Altitude (m.a.s.l)	2240
Annual rainfall (mm)	1230
Soil order	vertisol
pH	6.0
Organic matter (%)	1.6
NC(%)	0.17
P (PPM bray II)	1.8

Source: Wuletaw Tadesse, 1998

Appendix 3. Mean values of 14 morphological characters on 50 grasspea populations

Population	C	D	E	F	G	H	I	J	K	L	M	N	O	P
46003	9	101	49	142	3	335	3	8	2	2	2	147	76	57
46008	9	104	55	141	3	288	3	8	2	2	2	152	62	42
46012	9	119	56	145	3	462	3	8	2	2	2	228	104	44
46016	10	104	50	140	3	327	3	8	2	2	2	145	67	42
46020	9	103	52	140	3	344	3	9	2	2	2	159	77	46
46023	10	103	54	138	3	396	3	7	2	2	2	155	72	85
46024	10	103	54	141	3	486	3	8	2	2	2	219	115	48
46027	9	104	55	142	3	361	3	8	2	2	2	158	77	44
46030	10	111	61	145	2	328	3	8	2	2	2	179	70	36
46033	9	122	58	144	3	412	3	9	2	2	2	197	93	43
46034	9	116	46	143	2	306	3	10	2	2	2	164	80	48
46035	9	106	49	145	2	307	3	9	2	1	3	149	73	48
46042	9	112	56	144	3	477	3	8	2	2	2	206	86	46
46044	10	118	57	147	3	445	3	8	2	2	2	184	86	48
46049	10	117	59	151	3	509	3	8	2	2	2	220	00	44
46050	9	119	62	144	3	386	3	7	1	2	2	159	95	42
46077	10	109	62	152	3	454	3	8	2	3	2	292	98	41
46099	10	106	52	141	3	389	3	8	2	2	3	195	93	46
46100	8	113	44	134	3	301	3	9	2	2	3	144	73	47
46106	9	118	50	142	2	333	3	9	2	2	2	172	82	48
46110	10	194	42	130	3	334	3	8	2	2	2	123	65	52
46111	10	117	44	140	3	348	3	9	2	1	3	168	93	54
207493	11	121	52	148	3	517	3	8	2	2	2	259	107	43
207496	9	94	40	127	3	200	3	9	2	2	2	86	45	51
207497	8	104	45	136	3	282	3	9	2	2	2	143	79	53
207499	9	122	57	145	3	410	3	10	2	2	2	186	92	47
207566	8	93	41	130	2	198	3	8	2	2	2	87	42	40
207567	9	110	52	143	3	354	3	9	2	2	2	159	82	50
208449	9	107	55	142	3	422	3	8	2	2	2	206	95	46
211511	8	94	46	135	2	304	3	8	2	2	2	121	67	54
215247	11	120	52	140	3	504	3	8	2	2	2	229	116	48
219945	8	95	44	135	2	243	3	8	2	2	2	115	55	48
219946	10	114	46	142	2	481	3	9	2	1	2	209	104	50
219949	9	115	49	141	2	435	3	10	2	2	2	193	91	45
219959	9	101	49	135	3	291	3	10	2	2	2	145	79	44
219952	9	122	48	142	2	395	3	9	2	2	2	205	95	45
220118	10	107	52	148	2	464	3	9	2	2	2	226	105	44
226001	9	110	57	142	3	385	3	8	2	1	2	182	87	45
226006	8	115	51	135	3	355	3	9	2	2	2	182	90	46
226010	9	122	52	140	3	345	3	12	2	2	2	177	89	64
223219	9	105	39	135	3	315	3	9	2	2	2	141	79	56
226014	9	120	55	165	3	364	3	9	2	2	2	217	90	43
226019	11	121	57	143	3	530	3	8	2	2	2	231	114	49
228495	9	103	61	145	3	334	3	8	2	2	2	166	76	46
236711	8	122	57	150	3	441	3	8	2	2	2	257	87	42
46106	10	123	73	156	3	513	3	7	1	2	2	289	84	35
236686	7	106	60	148	3	253	3	7	1	1	2	107	55	53
236705	12	125	68	161	3	670	3	8	2	1	2	374	114	30
236708	11	110	71	156	3	675	3	7	2	2	2	303	127	42

* C = number of primary branches /plant, D= plant height, E = days to flowering, F = days to maturity, G = flower color, H = pods/plant, I = seeds/pod, J = 100 seed weight, K = seed size, L = pattern of testa, M = cotyledon color, N= biomass, O = seed yield /plant, P = harvest index

Appendix 4. Correlation among 9 different characters of grasspea in Gojam regionCharact

	Bim	DF	DM	HI	NPB	NPP	PLH	SPP	SW	SYP
Bim	0.026	0.410**	-0.093	0.397**	0.760**	0.482**	0.164*	0.038		0.824**
DF		0.368**	-0.068	0.163*	0.053	-0.089	0.196*	-0.051		-0.057
DM			0.062	0.200**	0.489**	0.528**	0.447**	0.087		0.374**
HI				-0.136	0.170*	0.128	0.058	-0.103		0.328**
NPB					0.387**	0.087	0.139	0.063		0.314**
NPP						0.505**	0.207**	-0.023		0.831**
PLH							0.294**	-0.017		0.483**
SPP								-0.03		0.153*
SW										-0.033
SYP										

Appendix 5. Correlation among 9 different characters of grasspea in Gondar region

Characters	Bim	DF	DM	HI	NPB	NPP	PLH	SPP	SW	SYP
Bim	0.026	0.41**	-0.093	0.397**	0.76**	0.482**	0.164*	0.038		0.824**
DF		0.368**	-0.068	0.163*	0.053	-0.89	0.196*	-0.51		-0.057
DM			0.062	0.200**	0.489**	0.528**	0.447**	0.087		0.374**
HI				-0.136	0.170*	0.128	0.058	0.025		0.038
NPB					0.387**	0.087	0.139	0.063		0.314**
NPP						0.505**	0.207**	-0.023		0.831**
PLH							0.294**	-0.017		0.483**
SPP								-0.03		0.13*
SW										-0.033
SYP										

Appendix 6. correlation among 9 different characters of grasspea in Shoa region

Characters	Bim	DF	DM	HI	NPB	PP	PLH	SPP	SW	SYP
Bim	0.172**	0.485**	-0.011	0.372**	0.803**	0.576**	0.308**	0.033		0.784**
DF		0.326**	-0.055	0.119**	0.155**	0.090**	0.194**	-0.015		0.086**
DM			0.136**	0.194**	0.518**	0.604**	0.465**	0.044		0.455**
HI				0.004	0.166**	0.111**	0.110**	-0.039		0.207**
NPB					0.397**	0.232**	0.166**	0.017		0.352**
NPP						0.589**	0.309**	0		0.787**
PLH							0.379**	0.005		0.557**
SPP								-0.007		0.298**
SW										-0.003
SYP										

Appendix 7. Correlation among 9 different characters of grasspea in Tigray region

Charac	Bim	DF	DM	HI	NPB	NPP	PLH	SPP	SW	SYP
Bim	0.118	.474**	.199**	.406**	.885**	.668**	.316**	0.075	.725**	
DF		.228**	-0.035	0.008	0.116	.128*	.215**	-0.081	0.068	
DM			0.327**	0.037	.443**	.636**	.434**	0.067	.359**	
HI				0.093	.277**	.298**	.221**	0.055	.302**	
NPB					.411**	.245**	0.075	-0.043	.294**	
NPP						.245**	0.075	0.085	.686**	
PLH							.416**	0.092	.485**	
SPP								-0.015	.214**	
SW									0.064	
SYP										

Appendix 8. Correlation among 9 different characters of grasspea in Wollo region

	Bim	DF	DM	HI	NPB	NPP	PLH	SPP	SW	SYP
Bim		0.116	.473**	.225**	.340**	.847**	.649**	.356**	0.035	.950**
DF			0.111	-0.096	0.02	0.116	0.028	0.034	0.012	0.068
DM				0.342**	0.210**	0.471**	0.618**	0.421**	0.052	0.490**
HI					0.214**	0.323**	0.275**	0.265**	-0.029	0.447**
NPB						0.387**	.289**	.192**	-0.049	0.347**
NPP							0.639**	0.316**	0.049	0.847**
PLH								0.432**	0.1	0.654**
SPP									0.063	0.387**
SW										0.026
SYP										

Appendix 9. Percentage of phenotypic classes for each population and region

Acc.no	leaflet size			flower color		seed size			seed color				pattern of testa			
Gojam	S	M	L	B	P	R	A	B	C	O	G	Br	Bl	1	2	3	4
46012	24	79	21	0	100	0	0	33	63	4	63	4	33	0	21	38	33	8
46030	24	96	4	0	58	42	0	4	92	4	33	13	50	4	25	50	17	8
46042	24	83	13	4	100	0	0	38	58	4	54	4	38	4	8	71	13	8
46044	24	92	8	0	96	4	0	25	67	8	71	0	25	4	4	67	21	8
46049	24	79	17	4	67	33	0	46	46	8	58	0	38	4	0	58	42	0
46050	24	79	21	0	83	13	4	63	37	0	46	0	50	4	13	54	33	0
46073	24	83	17	0	96	4	0	13	83	4	33	0	46	21	4	42	46	8
208449	24	63	29	8	83	17	0	21	75	4	54	0	46	0	13	54	29	4
226019	24	67	29	4	88	12	0	21	79	4	33	0	58	9	4	58	29	9
228495	24	87	13	0	92	8	0	42	46	12	54	0	42	4	13	71	12	4
236711	24	67	33	0	92	4	4	17	71	12	38	4	50	8	8	58	25	9
236686	24	92	8	0	100	0	0	33	63	4	67	0	29	4	8	50	42	0
236701	24	79	21	0	67	33	0	8	84	8	67	0	33	0	71	8	21	0
Region	312	80	18	2	87	13	0	28	66	6	52	2	41	5	15	52	28	5
Gonder																		
46033	24	79	17	4	87	13	0	8	50	42	33	0	59	8	17	50	29	4
207493	24	63	29	8	92	4	4	33	67	0	54	8	34	4	0	63	25	12
207499	24	38	54	8	83	17	0	4	75	21	58	17	25	0	8	58	17	17
226014	24	88	12	0	87	13	0	8	71	21	50	8	38	4	8	58	26	8
46107	24	71	29	0	96	4	0	63	37	0	67	8	17	8	21	54	17	8
236705	24	79	17	4	80	16	4	25	75	0	79	0	17	4	4	84	4	8
236708	24	96	4	0	96	4	0	50	46	4	63	4	29	4	8	75	9	8
Region	168	73	23	4	88	11	1	27	61	12	58	6	31	5	9	63	18	10
Shoa																		
46003	24	75	21	4	79	21	0	25	67	8	46	4	50	0	4	84	4	8
46008	24	63	29	8	92	8	0	21	75	4	42	4	46	8	8	67	21	4
46016	23	78	22	0	91	9	0	13	87	0	43	4	49	4	17	48	26	9
46020	24	67	33	0	100	0	0	4	88	8	54	8	38	0	4	75	13	8
46023	24	79	17	4	92	8	0	42	50	8	50	0	46	4	17	54	21	8
46024	24	71	29	0	80	20	0	25	71	4	46	4	46	4	13	70	13	4
46027	24	88	12	0	92	8	0	13	83	4	46	0	42	12	8	63	17	12
Region	167	75	23	2	90	10	0	20	75	5	47	3	44	6	10	64	18	8
Wollo																		
46034	24	79	21	0	67	33	0	0	75	25	58	4	38	0	0	58	29	13
46035	24	88	12	0	71	21	8	21	71	8	84	0	8	8	13	71	8	8
46099	24	88	12	0	83	17	0	17	75	8	50	8	38	4	4	54	17	25

46100	24	88	12	0	88	12	0	8	75	17	63	0	37	0	0	79	13	8
46106	24	79	21	0	46	50	4	13	67	20	46	4	50	0	4	80	8	8
4611	24	54	46	0	88	12	0	8	75	17	75	4	21	0	4	67	4	25
215247	24	67	33	0	96	4	0	13	83	4	58	4	38	0	0	88	8	4
226001	24	88	12	0	100	0	0	42	58	0	71	8	21	0	17	58	25	0
226006	24	88	12	0	79	17	4	13	67	20	54	8	38	0	4	63	29	4
226010	24	79	17	4	79	21	0	13	83	4	42	4	54	0	4	75	8	13
Region	240	80	20	0	79	19	2	15	75	10	60	5	34	1	5	69	15	11
Wellega																		
46110	24	83	17	0	88	12	0	33	59	8	54	4	34	8	0	50	29	21
211511	24	100	0	0	50	50	0	46	54	0	46	8	46	0	4	63	33	0
Region	48	92	8	0	69	31	0	40	56	4	50	6	40	4	2	56	37	5
Tigray																		
207496	24	79	21	0	88	12	0	4	88	8	38	12	50	0	12	63	13	8
207497	24	96	4	0	100	0	0	4	0	96	4	67	29	0	34	4	54	8
207556	24	96	4	0	75	25	0	13	79	8	38	0	58	4	4	79	17	0
207567	24	83	17	0	88	12	0	8	75	17	29	13	50	8	8	46	46	0
219945	24	83	17	0	29	71	0	29	67	4	46	12	42	0	13	71	12	4
219946	24	46	54	0	38	62	0	4	79	17	67	0	33	0	4	92	4	0
219949	24	83	17	0	67	33	0	4	54	42	42	0	58	0	4	67	29	0
219950	24	79	21	0	83	17	0	4	58	38	38	0	54	8	13	54	29	4
219952	24	79	21	0	75	25	0	4	67	29	42	8	46	4	8	88	4	0
223219	24	96	4	0	88	12	0	79	21	0	46	0	54	0	0	92	8	0
Region	240	82	18	0	73	27	0	15	59	26	39	11	48	2	11	65	21	3
Eritrea																		
220118	24	83	17	0	75	25	0	12	75	13	38	4	58	0	8	67	25	0
Ethiopia	1199	79	19	2	83	17	0	23	65	12	50	5	41	4	10	61	22	7

* leaflet size (S = small, M = medium, L = large); flower color (B = Blue, P = pink, R = red); Seed size (A= small, B= medium, C = large seeded); Seed color (O = olive, G = gray, Br = brown, Bl = black); pattern of testa (1 = spotted, 2 = dotted, 3 = mottled, 4 = plain)

Appendix 10. Mean ODAP and Protein contents for 50 grasspea populations

POPULATION	ODAP(%)	PROTEIN(%)
46003	.5750	30.92
46008	.3900	29.39
46012	.4140	29.19
46016	.5910	28.00
46020	.3760	26.50
46023	.5890	28.11
456024	.5650	29.28
46027	.4540	25.98
46030	.5070	23.93
46033	.4440	28.13
46034	.5330	30.39
46035	.4810	27.62
46042	.3630	28.95
46044	.3720	28.12
46049	.3470	29.14
46060	.3700	29.23
46077	.4120	29.28
46099	.4200	28.32
46100	.4890	29.31
46106	.4100	27.60
46110	.4210	29.93
46111	.4300	29.77
207493	.5520	30.32
207496	.5700	27.71
207497	.3130	28.48
207499	.4470	26.42
207566	.6220	29.38
207567	.5080	28.90
208449	.5330	23.95
211511	.4210	27.62
215247	.5260	26.21
219945	.4240	29.39
219946	.4610	31.94
219949	.4340	30.25
219950	.4630	31.04
219952	.5380	30.41
220118	.3550	27.63
226001	.4500	29.98
226006	.3500	28.52
226010	.4340	31.28
223219	.5310	28.32
226014	.4500	28.36
226019	.5780	30.43
228495	.5390	30.24
236711	.4760	29.62
46106	.4510	26.16
236686	.5820	26.32
236705	.3690	26.66
236708	.5280	31.36
236701	.4780	30.89

Aappendix 11. ODAP content per individual plants from 50 grasspea populations

NO	ENTR	Mg ODAP/100G SEED	NO	ENTRIES	Mg ODAP/100 G SEED
1	46003R1-1	500	40	207566R1-7	562
2	46008R1-3	442	41	207567R1-1	400
3	46020R1-1	398	42	208449R1-4	497
4	46023R1-1	837	43	211511R1-2	311
5	46024R1-7	916	44	215247R1-2	636
6	46027R1-8	725	45	219946R1-8	429
7	46030R1-2	489	46	219946R1-1	526
8	46030R1-4	654	47	219949R1-2	379
9	46034R1-2	662	48	219952R1-1	547
10	46035R1-5	646	49	219952R1-5	458
11	46044R1-6	421	50	219956R1-7	366
12	46049R1-5	429	51	220118R1-5	288
13	46050R1-7	379	52	223219R1-8	549
14	46073R1-7	364	53	226001R1-1	293
15	46100R1-8	453	54	226006R1-5	573
16	46106R1-8	620	55	22010R1-8	473
17	46110R1-5	531	56	226014R1-7	484
18	46111R1-5	453	57	226019R1-6	675
19	46111R1-7	416	58	236711-R1-8	685
20	207493R1-5	432	59	46107-R1-1	324
21	207497R1-5	188	60	236686-R1-6	366
22	207499R1-4	455	61	236705-R1-8	455
23	207566R1-3	408	62	236708-R1-4	526
24	236708-R1-4	526	63	46110R2-3	280
25	236701-R1-1	494	64	207493R2-3	589
26	46003R2-5	343	65	207496R2-2	468
27	46008R2-8	201	66	207497R25	340
28	46012R2-4	369	67	207499R2-5	311
29	46012R2-5	411	68	207567R2-8	468
30	46016R2-2	309	69	208449R2-8	617
31	46020R2-8	238	70	211511R2-5	426
32	46023R2-6	330	71	215247R2-5	458
33	46024R2-4	330	72	219945R2-7	398
34	46027R2-3	149	73	219946R2-5	429
35	46030R2-4	379	74	219950R2-7	382
36	46033R2-2	199	75	220118R2-3	249
37	46073R2-2	327	76	223219R2-6	492
38	46073R2-2	358	77	226001R2-8	489
39	46042R2-6	337	78	226006R2-7	275

40	46042R2-8	330	79	226010R2-7	272
80	46044R2-8	233	117	226014R2-5	269
81	46049R2-4	303	118	228495R2-5	309
82	46050R2-4	424	119	236711-R2-4	633
83	46099R2-5	296	120	46107-R2-2	330
84	46100R2-6	624	121	236686-R2-3	746
85	46106R2-3	199	122	236705-R2-4	275
86	236705-R3-6	306	123	46110R3-5	453
87	236708-R2-1	403	124	46111R3-1	421
88	46003R3-2	882	125	46106R3-6	411
89	46008R3-6	528	126	207493R3-7	636
90	46012R3-6	463	127	207496R3-7	696
91	46016R3-3	662	128	207496R3-3	547
92	46016R3-4	803	129	207497R3-3	413
93	46020R3-1	494	130	207499R3-5	576
94	46023R3-7	602	131	207566R3-5	897
95	46024R3-2	450	132	207567R3-6	657
96	46027R3-5	445	133	208449R3-7	487
97	46033R3-1	680	134	211511R3-1	528
98	46033R3-3	453	135	219952R3-3	610
99	46034R3-4	542	136	215242R3-7	484
100	46034R3-6	455	137	219945R3-6	510
101	46035R3-7	471	138	219946R3-7	691
102	46042R3-7	424	139	219949R3-7	570
103	46044R3-8	463	140	219950R3-8	607
104	46050R3-6	408	141	223219R3-2	552
105	46083R3-3	515	142	226001R3-2	570
106	46099R3-1	468	144	226010R3-8	557
107	46099R3-3	497	143	226014R3-2	599
108	46100R3-1	392	144	226019R3-1	623
109	228495R3-3	623	145	219949R2-7	353
110	236711-R3-7	471	146	226019-R2-5	437
111	46107-R3-4	633	147	220118-R3-6	528
112	236686-R3-5	547	148	226006R3-4	204
113	236708-R3-5	657			
114	236701-R3-5	515			
115	236701-R3-8	426			
116	46049R3-2	309			

Appendix. 12 Number of alleles in each locus for the ten grasspea populations

POP1 208449

AAT-1 AA:04 BB:21
AAT-2 AA:20 AB:05
EST-1 AA:09 BB:13
EST-2 AA:19
ACP-1 AA:23
ACP-2 BB:04 AB:21
ACP-3 AB:07 BB:16 AA:02

POP2 207499

AAT-1 AA:06 BB:19
AAT-2 AA:25
EST-1 AA:12 BB:03 AB:01
EST-2 CD:01 DD:03 AD:04 AC:01
AB:03 BD:03 AA:01
ACP-1 AA:16
ACP-2 BB:05 AB:11
ACP-3 BB:11 AA:05

POP3 46035

AAT-1 AA:03 BB:22
AAT-2 BB:18 AB:07
EST-1 AA:20 BB:04
EST-2 AA:01
ACP-1 AA:25
ACP-2 BB:05 AB:03 AA:15
ACP-3 AB:04 BB:17 AA:02 CC:02

POP4 219945

AAT-1 AA:01 BB:24
AAT-2 AA:16
EST-1 AA:17 BB:06
EST-2 AB:19 BB:05
ACP-1 AA:15 BB:02
ACP-2 AB:25
ACP-3 AB:08 BB:15 AA:02

POP5 46024

AAT-1 AA:02 BB:23
AAT-2 AA:25
EST-1 AA:23 BB:02
EST-2 AB:15 BB:10
ACP-1 AA:21 BB:04
ACP-2 AB:25
ACP-3 AB:12 BB:13

POP6 219950

AAT-1 AA:16
AAT-2 AA:16
EST-1 AA:07 BB:08
EST-2 AB:04 BB:12
ACP-1 AA:16
ACP-2 AB:12 AA:04
ACP-3 AB:06 BB:10

POP7 226001

AAT-1 AA:11
AAT-2 AB:05 BB:11
EST-1 AA:07 BB:07
EST-2 AB:07 BB:09
ACP-1 AA:16
ACP-2 AB:15 AC:01
ACP-3 AB:07 BB:09

POP8 46027

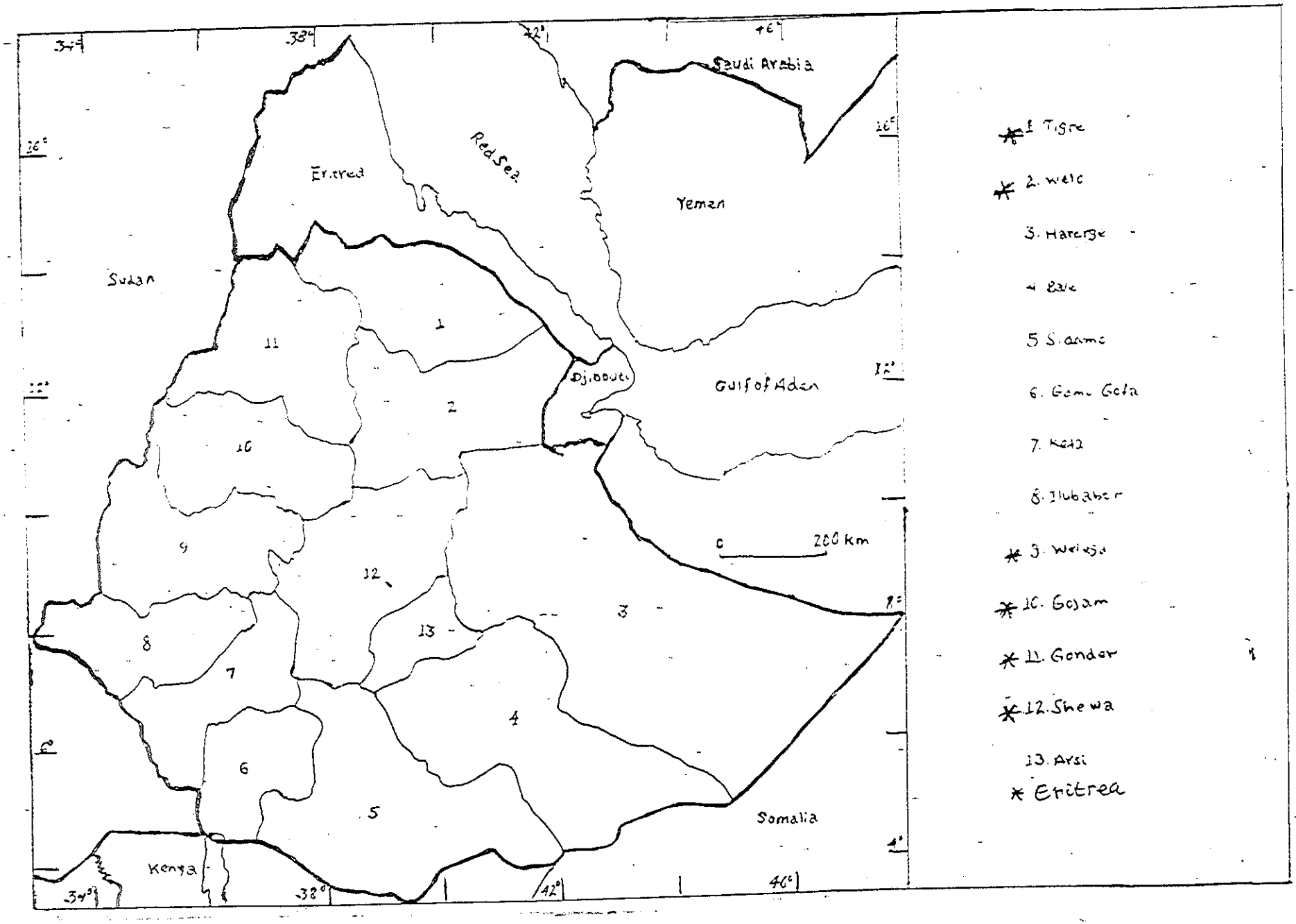
AAT-1 AA:02 BB:14
AAT-2 AA:16
EST-1 AA:11 BB:05
EST-2 AB:01
ACP-1 AA:16
ACP-2 AA:12 BB:04
ACP-3 AB:06 BB:10

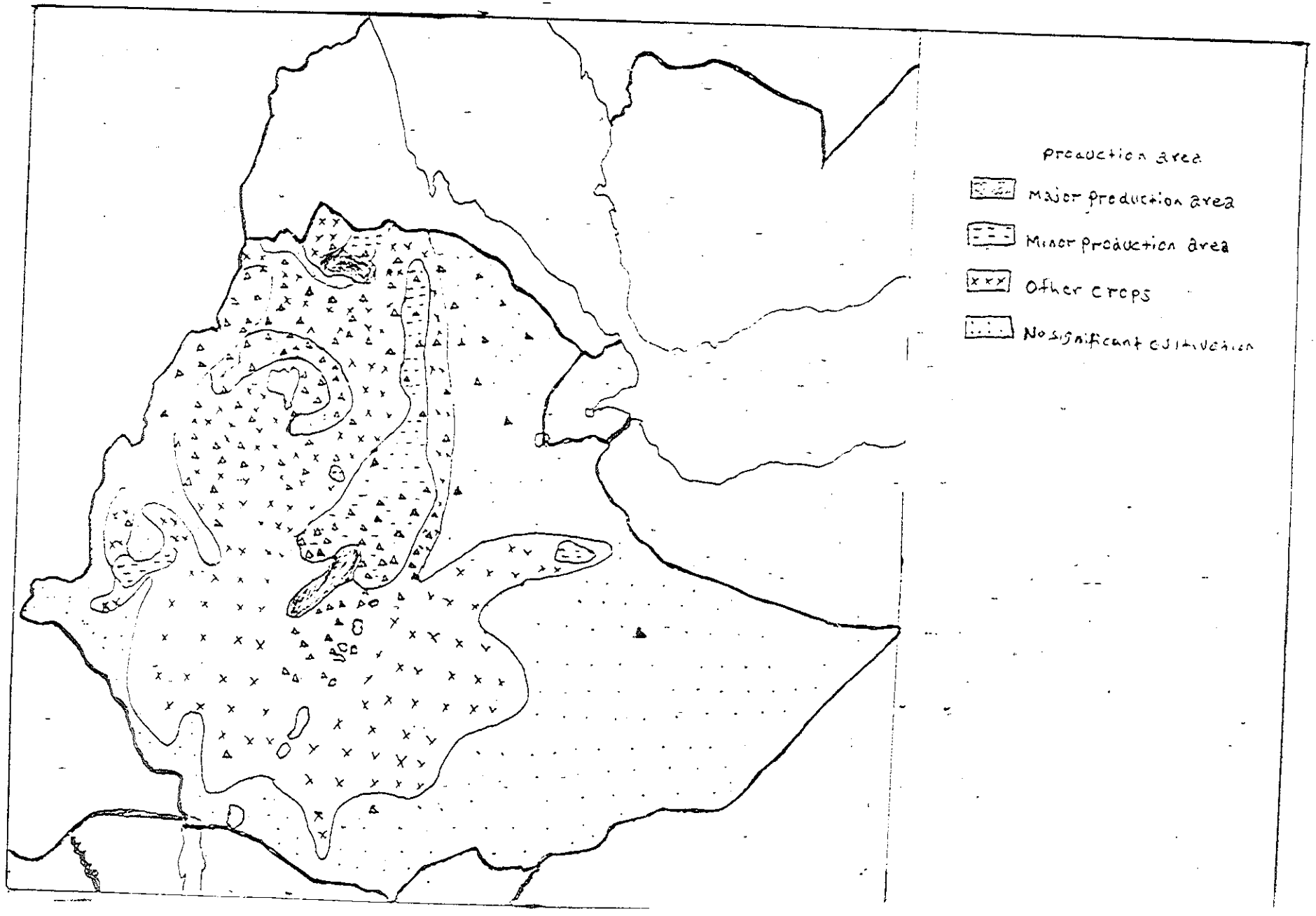
POP9 236711

AAT-1 AA:01 BB:15
AAT-2 AA:16
EST-1 AA:13 BB:03
EST-2 AB:01
ACP-1 AA:16
ACP-2 AA:01 BB:15
ACP-3 AB:03 BB:13

POP10 236705

AAT-1 AA:01 BB:15
AAT-2 AB:03 BB:13
EST-1 AA:04 BB:12
EST-2 CD:02 BC:07 AD:04 DD:01
BD:02
ACP-1 AA:16
ACP-2 AA:07 AB:09
ACP-3 AA:06 BB:10


Saudi Arabia
Eritrea
Red Sea
Yemen
Sudan
Djibouti
Gulf of Aden
Kenya
Somalia
0 200 km
1
2
3
4
5
6
7
8
9
10
11
12
13
1. Tigre
2. Welo
3. Hararge
4. Bale
5. Sidamo
6. Gamo Gofa
7. Kefa
8. Illubabor
9. Welega
10. Gojam
11. Gondar
12. Shewa
13. Arsi
Eritrea


Production area
Major production area
Minor production area
Other crops
No significant cultivation

Appendix 15 Standard Curve