GENETIC VARIABILITY BASED ON ISOZYME ANALYSIS IN NOUG (*Guizotia abyssinica)* POPULATIONS COLLECTED FROM GOJAM AND WELO, ETHIOPIA

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By

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Table of contents

List of Tables

List of Figures

Abstract

Isozyme electrophoresis was used to assess genetic variability in twelve populations of Guizotia abyssinica collected from Gojam and Welo regions. Three enzyme systems namely, phosphoglucose isomerase (PGI; E.C.5.3.1.9), ß-esterase (ß-EST; E.C.3.1.1.-), and aspartate aminotransferase (AAT; E.C.2.6.1.1.) were studied. A total of 17 alleles were observed from eight genetic loci for the three enzyme systems from which five loci were polymorphic. The mean percentage polymorphic loci (P_P), the mean number of alleles per locus (A_P), the mean observed heterozygosity (H_{op}) and the mean expected heterozygosity (H_{ep}) showed the range 37.5-62.5%, 1.625-1.875, 0.1875-0.3375 and 0.1901-0.2599, respectively. The mean population differentiation (F_{ST}) estimated was 0.2117. The averages of mean gene diversities of Gojam and Welo populations pooled separately were 0.2307 and 0.2064, respectively. The genetic distance calculated for each pair wise comparison ranged from 0.0012-0.2346. The results indicate the presence of high genetic variability within and between populations of Guizotia abyssinica. The results also show higher level of within population variability in Gojam than Welo populations. Dendrogram drawn based genetic distance revealed some level of association between populations of the same region.

1. Introduction

1.1 Taxonomy and names of *Guizotia abyssinica*

The genus *Guizotia* belongs to the family Compositae, tribe Heliantheae, subtribe Coreopsidinae. There are six species commonly referred to within the genus *Guizotia* of which five are native to Ethiopia. The six species are *G. abyssinica* (L.f.) Cass.; *G. scabra* (Vis.) Chiov. subsp. *scabra, G. scabra* (Vis.) Chiov. subsp. *schimperi* (Sch.Bip.) Baagoe; *G. arborescens* I. Friis; *G. reptans* Hutch.; *G. villosa* Sch.Bip. and *G. zavattarii* Lanza. (Baagoe, 1974). *G. reptans* is not present in Ethiopia. There are also other populations within the genus *Guizotia,* such as the Chelelu and Ketcha populations whose taxonomic status is not yet settled (Kifle Dagne, 1995) and *G. bidentoides* Oliver and Hiern (Murthy, 1990 cited in Getinet Alemaw and Sharma, 1996). *G. abyssinica* (noug or niger) is a cultivated crop while the rest of the taxa are weedy or wild plants (Kifle Dagne, 1994a).

The earliest name given to *G. abyssinica* was *Verbesina olifera* by Buchoz in 1775. However, this name is invalid because the binnary system was not used throughout Bouchoz's work. The first valid name was *Polymnia abyssinica* L.f. from 1781. The plant is known by many other synonyms: *Verbesina sativa* Roxb. ex Sims, *Polymnia frondosa* Bruce, *Parthenium luteum* Spr., *Heliopsis platyglossa* Cass., *Jaegeria abyssinica* Spr., *Guizotia oleifera* DC., *Veslingia scabra Vis.*, *Ramtilla oleifera* DC. In spite of all these names, the name *Guizotia* Cass was conserved by the Vienna congress in 1905 and the name *Guizotia abyssinica* (L.f.) Cass. was proposed as a type species of *Guizotia* Cass (Baagoe, 1974; Getinet Alemaw and Sharma, 1996).

1.2 Cytology and karyotypes of *Guizotia abyssinica*

G. abyssinica, like all other species in the genus, has 2n = 30 chromosomes (Hiremath and Murthy, 1992). Baagoe (1974) and Kifle Dagne (2001a) proposed the basic chromosome number x = 5 for the genus *Guizotia*. Kifle Dagne (2001a) considered the currently known *Guizotia* species as probably ancient hexaploids with 2n = 6x =30. Meiotic analysis of *G. abyssinica* revealed 15 regular bivalents at diakinesis and metaphase 1 of meiosis (Hiremath and Murthy, 1992; Murthy *et.al.*, 1993). However, Hiremath and Murty (1992) recorded quadrivalent and monovalent formation in some populations of *G. abyssinica.*

The chromosomes of *G. abyssinica* have characteristics such as small size, centromeric positions of either m or sm types and poor constitutive heterochromatin regions. Thus, it is difficult to identify homologous chromosomes on the basis of their size, centromeric position and C-banding technique. A maximum number of eight satellited chromosomes were observed on late prophase and prometaphase stages and all the satellited chromosomes were associated with the nucleolus. Also a maximum of four, six, and seven nucleoli were observed in microspores, somatic interphase and telophase nuclei, respectively. This suggested the presence of eight major nucleolus organizing regions in the diploid complement. Thus, it was deduced all the eight satellited chromosomes of *G. abyssinica* contain an active nucleolus organizer region (Kifle Dagne and Heneen, 1992). In addition, Kifle Dagne *et.al.* (2000) reported the presence of eight rRNA gene loci for *G. abyssinica*, which correlates with the presence of eight satellited chromosomes in the diploid complement of this species as reported by Kifle Dagne and Heneen (1992).

G. abyssinica is cross-compatible with three wild taxa: *G. scabra* ssp *scabra*, *G. scabra* ssp *schimperi* and *G. villosa*, and sexual hybrid can be made easily between the cultivated and the wild taxa. This shows that gene transfer is possible through introgressive hybridization. However, crosses were less successful when *G. abyssinica* served as female than as male parent. Data on interspecific crossability and on meiosis of interspecific hybrids indicate that G. *abyssinica* is more closely related to *G. scabra* ssp *schimperi* than *G. scabra* ssp *scabra* and *G. villosa* (Kifle Dagne, 1994b).

1.3 Origin of *Guizotia abyssinica*

Results from crossability and meiosis in hybrids indicate that the chromosomes of *G. scabra* ssp *schimperi*, *G. scabra* ssp *scabra* and *G. villosa* share a large degree of homology and that these taxas differ from each other by paracentric inversions (Kifle Dagne, 1994a). Symmetrical karyotypes are considered to be correlated with primitive morphological characters and asymmetrical karyotypes as derived ones. The karyotype of G. abyssinica is symmetrical which could indicate that it is primitive taxa in the genus (Hiremath and Murthy, 1992). However, in *Guizotia* characteristics of the karyotype should be used in conjunction with other characteristics of organisms such as results from morphology and hybridization to obtain reliable information about the direction of evolution (Kifle Dagne, 1994a).

Baagoe (1974) suggested that the niger crop was derived from G. *scabra* most probably from *G. scabra* ssp. *schimperi* because they share several common morphological features. Also the karyotypes of the two species are alike and uniform which is inferred from similar chromosome size, absolute chromosome length, the type and number of SAT chromosomes and satellite size observed in both taxa (Hiremath and Murthy, 1992). However, Kifle Dagne (1995) reported that *G. scabra* ssp. *schimperi* has relatively smaller chromosomes, which results in the differences in DNA amounts between the two taxa and that the species karyotypes are similar but not identical.

The F1s of *G. abyssinica* x *G. scabra* ssp. *schimperi* showed: high percentage (81%) of bivalents in pollen mother cells, high pollen and seed fertility which indicated that the genomes of the two taxa are basically similar and fully homologous (Murthy *et.al.*, 1993). Also hybridization studies of Kifle Dagne (1994b) are in confirmity with this report. Thus, based on the information from karyotypes, hybridization and morphological characteristics, *G. abyssinica* might have originated from *G. scabra* ssp. *schimperi* through selection and further cultivation of a "large achened mutant" (Murthy *et.al.*, 1993).

The Chelelu population is closely related to *G. abyssinica* and *G. scabra* ssp. *schimperi* which is inferred from their chromosome morphology (Kifle Dagne, 1995). On the basis of chromosome morphology and F1 meiosis, the Chelelu population and *G. scabra* ssp. *schimperi* are closely related, and it was suggested by Kifle Dagne (1994a) that the latter was derived from the former or that they share an immediate common ancestor.

G. abyssinica was most probably originated in Ethiopia, and was introduced and naturalized in the Indian subcontinent through ancient trade between India and the Horn of Africa. Several facts could be taken as indications to strengthen this assertion. First, the present center of gene variation is found in Ethiopia. Second, all the wild *Guizotia* species except *G. reptans* are present in Ethiopia. Third, no wild relative of *G. abyssinica* is found in India (Baagoe, 1974; Kifle Dagne, 1994a).

Moreover, because *G. scabra* ssp. *schimperi* is native to Ethiopian highlands (it is called "mech", a common weed, in Ethiopia), it is assumed that *G. abyssinica* was domesticated in Northern Ethiopia as early as 3000 B.C. and that the crop was introduced to India, probably through trade routes, prior to the Christian era (Baagoe, 1974; Hiremath and Muthy, 1988).

1.4 Oil content of *Guizotia abyssinica*

G. abyssinica seed contains 30-50% oil with a mean of 41.3% (Kifle Dagne, 1994a). The suitability of any oil for a particular purpose is determined by its fatty acid composition. Regarding the fatty acid profile, *G. abyssinica* seed oil has linoleic acid (C18:2) as the principal fatty acid (65.7 -68.5 %, weight percent of total lipid). Oleic acid (C 18:1) was the second major unsaturated fatty acid (5.4-7.5 %). *G. abyssinica* contains two major saturated fatty acids [palmitic (9.6-10 %) and stearic (7.6-8.1 %)]. The above fatty acids represent 91-97% of the fatty acid present. Palmitoleic, linolenic, arachidic, eicosenoic, behenic, erucic and lignoceric acids constituted less than 1% each (Kifle Dagne and Jonsson, 1997).

The quality of an oil can also be determined from its content and composition of triacylglycerol (TAG) molecular species and sterol (ST). TAGs are the main components of vegetable oils and phytosterols have antioxidant property and have health benefits. The TAG structure of *G. abyssinica* Cass. seed oil contains high amounts of palmitic, oleic and linoleic acid. *G. abyssinica* has a high amount of sterol (phytosterol), which consists of 0.40%- 0.42%, weight percent of TL (total lipid content). ß-sitosterol, campesterol, stigmasterol, and D5-avenasterol comprising together about 90% of total ST content, while ß-sitosterol alone constitutes a half of the ST contents. This indicates that *G. abyssinica* seed oil is a good source of phytosterols (Ramadan and Morsel, 2002).

Dietary fats and oils, rich in linoleic acid, have been reported to prevent cardiovascular disorders such as coronary heart disease, atherosclerosis, as well as high blood pressure. Also linoleic acid derivatives serve as structural components of the plasma membrane and as precursors of some metabolic regulatory compounds (Vles and Gottenbos, 1989 cited in Kifle Dagne and Jonsson, 1997). Thus, the seed oil of *G. abyssinica* has good nutritional value because of its high linoleic acid content and its purity from undesirable fatty acids for human consumption (Kifle Dagne and Jonsson, 1997).

1.5 Economic importance and distribution of *Guizotia abyssinica*

G. abyssinica develops a pale yellow oil with a nutty taste, less fluid, clear and a pleasant odour. In Ethiopia, *G. abyssinica* is mainly cultivated for its edible oil. Besides cooking, the *G. abyssinica* seed is used as a human food. The oil of G*. abyssinica* can also be used for lighting, painting, soap-making, anointing and cleaning of machinery or as lubricant. The oil is used in birth control, cooked with spices, in the treatment of syphilis. *G. abyssinica* sprouts mixed with garlic and 'teg' (mead) are used to cure cough (Seegeler, 1983).

The meal remaining after oil extraction contains about 24% protein and 24% crude fiber in the Ethiopian varieties whereas the Indian varieties contain 30% protein and 17% crude fiber. The press cake of *G. abyssinica* can replace linseed cake in calves and groundnut cake in pigs without significant difference (Quinn and Myers, 2002). The *G. abyssinica* cake can also be used as manure or fuel. Agar medium prepared from *G. abyssinica* can be used to isolate a fungus, *Cryptococcus neoformans* (Sant) Vaill which causes a serious brain ailment. The fresh *G. abyssinica* plant is consumed by sheep while the seed is feed for cage birds in Europe and N. America (Seegeler, 1983).

In, Africa, *G. abyssinica* is largely found in the Ethiopian highlands, particularly west of the Rift Valley. *G. abyssinica* is also found in some areas in Sudan, Uganda, Zaire, Tanzania, Malawi and Zimbabwe, and the West Indies, Nepal, Bangladesh, Bhutan and India (Riley and Belayneh, 1989; Getinet Alemaw and Sharma, 1996).

G. abyssinica is an important oil crop in Ethiopia and parts of India. It provides 50-60% of the oilseed production in Ethiopia and 3% in India (Seegeler, 1983). In 2003/04 main ('meher') season in Ethiopia the total area cultivated with *G. abyssinica* was 281, 718 ha and from this area 11,895.5 tonnes of the crop were produced. In that same year, the productivity of *G. abyssinica* was 422 kg/ha (Central Statistical Authority, 2004).

1.6 Ecology and agronomy of *Guizotia abyssinica*

G. abyssinica is a crop of the cooler parts of the tropics. The major *G. abyssinica* - producing areas in Ethiopia are characterized by moderate temperature ranging between 16°C and 20°C during the growing season. In Ethiopia, *G. abyssinica* is cultivated at altitudes ranging from 500m to 2980 m but mainly grown in mid-altitude and highland areas (1700-2200m a.s.l.). It grows well in areas where rainfall is 1000 to 1300 mm per year (Seegeler, 1983)

G. abyssinica does not show a clear preference for particular soil types, as long as they are not very loose or very heavy. In Ethiopia it is usually sown in areas with a rather poor soil or on heavy clay soil under poor cultural conditions (Seegeler, 1983). It grows well at pH values between 5.2 and 7.3 (Getinet Alemaw and Sharma, 1996).

G. abyssinica develops aerenchymas in the stem (corms) where there is poor aeration in soil due to high waterlogged condition. The low oxygen supply in soil destines the development of underground organs which in turn would have made the evolution (development) of copious intercellular spaces inevitable to counteract the conditions of oxygen deficiency in the soil by diffusing gasses into the root system. Thus, *G. abyssinica* tolerates waterlogged soils and the poor oxygen supply in soil (Yohannes Petros, 1988).

G. abyssinica is a good precursor for crops and the crops following *G. abyssinica* show increased growth and yield with less weed infestation (Hadgu, 1972 personal communication through Endashaw Bekele). It could be due to a microorganism involved in mycorrhiza association (*Glomus macrocarpus*) (Seegeler, 1983) and a component from water extract of *G. abyssinica* (not identified yet) that inhibited germination of monocotyledonous weeds (Getinet Alemaw and Sharma, 1996).

Ethiopian *G. abyssinica* is classified into three types based on its maturity. These are: (i) Abat (medium to late maturity) *G. abyssinica* which is grown within altitudes of 1500 to 2500 m on heavy black clay waterlogged soils with adequate rainfall. It is grown in the mid-and high-altitude regions of Gojam, Gonder, Shoa and Wellega. It is planted in mid-May to early June and harvested in December. Abat *G. abyssinica* is higher yielding and has a longer growing season than 'bungne' and 'mesno' *G. abyssinica*, and the oil content of 'abat' types is also higher than that of 'bungne' types. (ii) Bungne (early maturing) *G. abyssinica* is grown in lowland and highland areas with low rain fall on shallow soils. It grows in Tigrai, lowlands of Welo and Abay Gorge. It is planted in July and harvested in October. Bungne types are low yielding and the seed has low oil content. (iii) Mesno (late but frost tolerant) *G. abyssinica* has growing season from September to February (Getinet Alemaw and Adefris Tekelewold, 1995; Getinet Alemaw and Sharma, 1996).

G. abyssinica seed yield has low response to nitrogen and phosphorous fertilizer and to drainage. Fertilizer and drainage promote luxurious vegetative growth but do not increase seed yield (Getinet Alemaw and Adefris Tekelewold, 1995). The plant is not adapted to fertilizer levels common for other crops. Such levels increased vegetative growth enormously and induced lodging resulting in a relative loss of seed yield of 37%. Lodging and shattering are the two most important causes of low seed yield in *G. abyssinica* (Seegeler, 1983).

There are few diseases and pests reported on *G. abyssinica* than other oilseed crops such as sunflower, sesame and linseed (Getinet Alemaw and Adefris Tekelewold, 1995). Dodder (*Cuscuta campestris*) is the major parasitic weed of *G. abyssinica* while the niger fly (*Dioxyna sororcula* and *Eutretosoma* spp) and black pollen beetles (*Meligethes* spp) are the most important insect pests of the crop. The following diseases are the most serious on *G. abyssinica*: niger blight (*Alternaria* sp.) and leaf spot (*Xanthomonas campestris* pv. *guizotiae*) (Seegeler, 1983; Getinet Alemaw and Sharma, 1996).

1.7 Biotechnological approaches for breeding of *Guizotia abyssinica*

G. abyssinica production in Ethiopia is mainly based on local landrace populations (Getinet Alemaw and Sharma, 1996). Getinet Alemaw and Adefris Tekelewold (1995) reported the presence of wide genetic variation in *G. abyssinica* for agronomic traits: days to flowering, days to maturity and plant height, which could be utilized for breeding of *G. abyssinica* varieties for certain climatic and soil conditions. They also reported the presence of significant variability in oil content of *G. abyssinica* that could be utilized for breeding high-oil-content *G. abyssinica* cultivars. Extensive basic research and breeding work including modern biotechnological approaches are needed to realize genetic improvement in this crop (Kifle Dagne, 2001b; Adefris Teklewold and Girma Mamo, 2002).

Developing self-compatible lines could be one of the target approaches for genetic improvement of *G. abyssinica* (Sileshi *et.al.*, 1999). *G. abyssinica* is a completely outcrossing species with sporophytic self-incompatibility system. Honey bees are the primary agent of cross pollination in *G. abyssinica* (Ramachandran and Menon, 1979). Self-incompatibility (SI) is a disadvantage in the breeding system of many crops and their improvement scheme when either free intercross or inbreeding is required. Self-incompatibility is one of the major problems which are associated with the process of screening of the national germplasm of *G. abyssinica* for improvement (Sileshi *et.al.*, 1999).

The self-incompatibility in *G. abyssinica* is mostly controlled by a single S-locus. Different types of relationships are observed by the S-locus alleles both in pollen and style. Additional S-locus allele or an independent S-locus (S-loci) is also suggested to be part of the SI system in this crop. The self-incompatibility system in *G. abyssinica* is characterized by the presence of two-and one-way incompatibility, reciprocal difference, and self-compatibility (Sileshi *et.al.*, 1999).

Different frequency of self-compatible genotypes both within a region and at interregional level are recorded which can provide an enormous diversity of self-compatible genotypes for breeding activities to establish self-compatible genotypes. However, considerable selection procedures are required before getting entirely self-compatible quality genotypes because, further crossing experiments have revealed self-incompatibility in the progeny of self-compatible parents (Sileshi *et.al.*, 1999).

Self-compatible lines, dwarfs and single-headed dihaploid plants of *G. abyssinica* have been produced from anther culture of *G. abyssinica* in India. These dihaploids can be used to develop homozygous mutant types and inbred lines in a short time (Sarvesh *et.al.*, 1993b, 1994a cited in Getinet Alemaw and Sharma, 1996).

The production of artificial autotetraploid plants in *G. abyssinica* have been reported, which can be used for the production of primary trisomics that allows further cytogenetic studies of this crop (Kifle Dagne, 2001b).

1.8 Isozyme analysis

Two general forms of protein data can be gathered simultaneously using electrophoretic methods. One is derived from isozymes, which are any of more than one form of the same enzyme encoded by different gene loci. The other data set consists of allozymes, which are any of more than one variant of an enzyme encoded by different alleles at the same gene locus (Murphy *et.al.*, 1996; Chamberlain, 1998a).

The primary observed evidence in studies of electrophoretic variation of isozymes in natural populations is coloured bands in a slab of starch gel (Gottlieb, 1977). They are revealed when enzymes from tissue extracts are subjected to electrophoresis in various types of gels and subsequently submerged in solutions containing enzyme-specific stains (Wendel and Weeden, 1989). The enzyme variants are separated because they have different net electrostatic charges. Their migration through the gel may also be differentially affected by their size or configuration. Following their physical separation, the enzymes are identified by a staining reaction based on their specific catalytic activities. The combination of electrophoresis and staining specificity makes it possible to distinguish particular enzymes among hundreds that may be present in a crude tissue extract (Gottlieb, 1977; Chamberlain, 1998a).

The relationship between observed phenotypes and unobserved genotypes is simpler and better understood for electrophoretic evidence. Allozymes are the biochemical consequence of the substitution, deletion, or addition of amino acids in the polypeptides that comprise the enzymes, and they can be distinguished if these changes affect their electrophoretic migration (Gottlieb, 1977). Since the amino acid sequence of a polypeptide is collinear to the nucleotide sequence of its coding structural gene locus, allozymes result from gene mutation. Thus, isozyme analysis which is an analysis of protein structure using electrophoresis is an analysis of a gene (Crawford, 1990).

A major advantage of isozyme analysis is that it is a codominant, single-locus genetic marker because it allows individuals to be identified as homozygous or heterozygous at a given locus (Hamrick, 1989; Parker *et.al.*, 1998). A second significant advantage is that isozyme analysis avoids the problem of convergence and functional correlation, as it allows systematic comparisons between products of genes which are homologous (have a common origin). This is due to the simpler relationship between amino acid sequence and nucleotide sequence (Weeden and Wendel, 1989). A third significant advantage is that electrophoresis evidence is precise and directly quantifiable in terms of the number and kinds of enzymes studied, permitting the amount of genetic information utilized to be stated exactly (Gottlieb, 1977).

In most cases, isozyme electrophoresis exhibit high penetrance and absence of pleiotropic and epistatic interactions. It allows comparisons with enzymes that are generally always present and little influenced by environmental factors (Weeden and Wendel, 1989). Another advantage is that problems of a priori character weighting do not occur with electrophoretic evidence because all enzymes examined are given equal value in similarity matrices or other methods of evaluating divergence (Gottlieb, 1977).

However, these advantages of isozyme analysis are offset by certain limitations. The first limitation is that enzymes extracted and subjected to electrophoresis are a tiny and probably non-representative sample of the total array of proteins present in an organism, and electrophoretic differences are only one kind of difference, and on the whole, a minor one, of the various differences that exist even between genetically related proteins (Weeden and Wendel, 1989). Underestimation of the actual amount of genetic difference between taxa is another problem of isozyme analysis. Due to the redundancy of the genetic code, only about 30% of the substitutions of nucleotides are expected to result in the substitution of amino acids that cause changes in electrophoretic mobility (Gottlieb, 1977).

A single mobility class on a gel may sometimes contain more than one enzyme (Murphy *et.al.*, 1996). This indicated that more weight be given to evidence of electrophoretic difference than to evidence of similarity. Electrophorectic evidence do not show whether the difference in mobility is due to a single nucleotide substitution or numerous changes in nucleotide sequence. Thus, it lacks information on the number of amino acid differences, or mutational steps, that cause differences in enzyme mobilities (Gottlieb, 1977).

Null alleles (those with reduced or no expression of a protein product) cause deviation from codominant expression of allozymes. The interpretation of null alleles is often ambiguous and requires confirmation by breeding studies (Kephart, 1990). Secondary isozymes (conformational isozymes), multiple forms of a single gene product that differ in secondary or tertiary structure, are also limitations of isozyme analysis. They can result from posttranslational processes or epigenetic events. Such alterations of mobility may lead to incorrect hypothesis about the number of loci encoding an enzyme system (Murphy *et.al.*, 1996).

In spite of its limitations, isozyme analysis can be utilized because the limitations are reasonably well defined and the direction of bias is generally known (Gottlieb, 1977). Isozyme as marker genes still continue to grow in value solving a variety of problems in population genetics, systematics, evolution and plant breeding (Weeden and Wendel, 1989; Crawford, 1990).

Isozyme analysis provides efficient, quantitative estimates of the amount of genetic variation within natural populations and the extent of genetic divergence among populations (Gottlieb, 1977). Isozyme electrophoresis is used to describe population structure, breeding structure and gene flow; to know species boundaries, and to document adaptive differences in allozymes; and to investigate phylogenetic relationships, rates of evolution, origin of polyploid plants and ploidy levels (Murphy *et.al.*, 1996).

The use of isozymes as markers is of considerable value in plant breeding. In plant breeding isozymes are used to quantify interpopulation and intrapopulation genetic diversity and to determine population structure of various crop species. The genetic diversity identified through isozyme analysis is important for breeding and crop improvement programmes (Getinet Alemaw and Sharma, 1996). Furthermore, it enables to detect the rate of erosion and irreversible loss of genetic variability and structure of crop varieties at the centers of genetic diversity which would facilitate the conservation of genetic diversity in landrace crop populations (Frankel, 1974; Mulatu Geleta, 2001).

2. Objectives

General objective

- To investigate genetic diversity of *Guizotia abyssinica* populations from Gojam and Welo.

Specific objectives

- To estimate and compare some genetic variability measures within populations of *G. abyssinica*.
- To estimate and compare some genetic variability measures between populations of *G. abyssinica*.
- To determine genetic distance values between populations of *G. abyssinica*.
- To compare the genetic variability of Gojam and Welo *G. abyssinica* populations.
- To indicate the enzyme systems that reveal polymorphic isozymes in *G. abyssinica*.
- To generate information for future research on noug at biochemical level.

3. Materials and Methods

3.1 Plant materials

A total of twelve populations from two regions (Gojam and Welo) were considered in the present study and a sample of ten individuals per population was studied (Table 1). All the seed samples were kindly provided by Ato Mulatu Geleta. The plant materials used for this study were embryonic leaves harvested from three day old seedlings which are grown in Petri dish at green house condition.

3.2 Enzyme extraction, electrophoresis and isozyme staining

Tissue extracts from embryonic leaves of three, five and seven days old seedlings were tested for quality of banding patterns but the embryonic leaves from three days old seedlings were used for isozyme analysis because of their better resolution. The leaves were ground in two drops of general extraction buffer comprising: 50 mL lithium-borate gel buffer, 37 mg potassium chloride, 10 mg magnesium chloride, 18mg disodium EDTA, 25 mg PVP, 0.5 mL Triton-X-100 and 2 mL ß-mercaptoethanol (Chamberlain, 1998a). During maceration the perspex extraction trays were kept on crushed ice to prevent denaturation of the enzymes. Crude enzyme extracts were absorbed onto filter paper wicks and loaded onto the starch gels. Horizontal electrophoresis was carried out in starch gels (11% mass/volume) at constant 70mA(250 V) until the 1% bromophenol blue tracker dye had migrated 8 cm from the origin.

The starch gel used for electrophoresis was prepared following the protocol of Chamberlain (1998a). First, 46.75 g of electrophoresis grade hydrolysed potato starch was suspended in 106.25 ml of gel buffer in Buchner flask. Another 318.75 ml of gel buffer was heated in a conical flask and boiled in microwave oven for five minutes. Then, the boiling buffer was added to the starch solution in the Buchner flask and the Buchner flask was shaked vigorously. The Buchner flask was heated over a medium- hot bunsen flame. While heating over the bunsen burner the Buchner flask was vacuumed for 40 seconds using a vacuum pump to remove any air bubbles. The starch mixture was quickly poured into the center of a glass gel mould. Finally, after cooling the gel at 4 ºC overnight, it was used for electrophoresis in the next morning.

Table 1. Localities of *Guizotia abyssinica* used in the present study.

Coll. Code	Number of individuals used	Altitude (m)	Wereda	Site of collection
F8	10	2420	Delanta & Dawunt	31 Km from Wegel Tena to Gashena, Welo
G8	10	1882	Guba Lafto	18 Km from Woldia to Lalibela road, Welo
C10	10	1845	Habru	8 Km from Mersa to Woldia, Welo
H7	10	1440	Jille and Tumuga	28 Km from Shewa Robit to Kombolcha, Welo
V7	10	1910	Kalu	14 Km from Kombolcha to Bati, Welo
A8	10	2045	Tewledere	4 Km from from Haik to Bistima, Welo
J19	10	1860	B/Dar Zuria	7 Km from B/Dar to Tis Isat, Gojam
L18	10	2460	Dejen	1 Km from Dejen to D/Markos, Gojam
O19	10	2440	Fagita-Lekuma	17 Km from Injibara to Dangla, Gojam
A17	10	2330	Mashakel	23 Km from D/Markos to Dangla, Gojam
D17	10	2050	Jabit-Tehinan	2 Km from F/Selam to Bure, Gojam
B19	10	2350	Yilma and Densa	62 Km from B/Dar to Mota, Gojam

Three enzyme systems were selected to identify potentially polymorphic isozymes for detailed analysis after a preliminary survey of seven enzymes: Acid phosphatase (ACP; E.C.3.1.3.2), Glucose-6-phosphate dehydrogenase (G6PDH; E.C.1.1.1.49), Isocitrate dehydrogenase (IDH; E.C.1.1.1.42), Leucine aminopeptidase (LAP; E.C.3.4.11.1.), phosphoglucose isomerase (PGI; E.C.5.3.1.9), ß-esterase (ß-EST; E.C.3.1.1.-), and aspartate aminotransferase (AAT; E.C.2.6.1.1.). The three enzyme systems selected to resolve isozymes were: PGI, ß-EST, and AAT.

Lithium- borate buffer system (pH 7.6) was used for all enzyme systems. The gel buffer for this system contains 0.042 M tris-base, 0.007 M anhydrous citric acid, 0.004 M lithium hydroxide and 0.025 M boric acid. The electrode buffer contains 0.039 M lithium hydroxide and 0.263M boric acid (Kephart, 1990).

After electrophoresis was completed, the gel was sliced and stained using specific staining recipes. The following staining recipes were used according to the protocols of Chamberlain (1998a), and Wendel and Weeden (1989). For AAT: 50 ml 0.1M Tris-HCl (pH 8.5), 18 mg α-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. For PGI: 50 ml 0.1 M Tris-HCl (pH 7.5), 20 mg disodium fructose-6-phosphate, 20 units glucose-6-phosphate dehydrogenase, 7 mg NADP, 12 mg MTT, 3 mg PMS and 0.5 ml 10% $MgCl_2$. 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% ß-napthyl acetate in acetone and 125 mg fast blue BB salt and 1 ml acetone.

After 30 minute incubation at 37 ºC the staining solution was poured off and the developed bands were fixed using glycerol: water (1:1 ratio). The isozyme data for AAT and PGI was scored immediately. For EST the fixed slice is stored at 4 ºC overnight and scored in the next morning.

3.3 Analysis of isozyme polymorphisms

Genetic interpretations of isozyme and allozyme variation patterns of the enzymes were based on three lines of evidence: (1) typical patterns of gene expression (number, spacing and intensity of enzyme bands) from other plants; (2) knowledge of active subunit composition of other homologous enzymes (i.e. whether they are monomers, dimers, etc) (Wendel and Weeden, 1989; Kephart, 1990; Chamberlain, 1998a); (3) considering isozyme genetic interpretation in related genera such as sunflower (Cronn *et.al.*, 1997; Carrera *et.al.*, 2002), *Coreopsis cyclocarpa* (Crawford and Bayer, 1981), and *Antennaria* species (Bayer and Crawford, 1986), where the information was available.

After scoring the bands were interpreted into a genotype on this basis, and entered into POPGENE version 1.31 (Yeh *et.al.*, 1999). Then the following measures of genetic variation and structure were calculated:

(i) Allele frequencies (A), both A_s (allele frequency calculated as a total for all the populations) and A_p (allele frequency calculated as means for the populations studied).

(ii) Percent polymorphic loci (P), both P_p (percent polymorphic loci calculated as the mean of the values obtained from each population) and P_S (percent polymorphic loci calculated as total for all populations).

(iii) Observed heterozygosity (H_o): measures proportion of observed heterozygotes.

(iv) Gene diversity or expected heterozygosity (H_e): measures genetic variability of a population which is equal to the probability of non-identity of two randomly chosen genes from a population. This probability is equivalent to the proportion of heterozygotes that would be expected under Hardy-Weinberg equilibrium (Nei and Roychoudhury, 1973; Nei, 1987). Both H_{ep} (mean gene diversity) and H_{es} (total genetic diversity) are calculated based on Nei's (1978) unbiased heterozygosity.

(v) Population differentiation (F_{ST}): measures the degree of genetic differentiation between populations, and gene flow (Nm, the number of migrants per generation) estimated from the estimate of F_{ST}. F_{ST} compares the ratio of the between population component of diversity to the total diversity (Nei, 1987; Chamberlain, 1998b).

(vi) The fixation index (F_{IS}): measures the deviation of genotypic frequencies from the Hardy-Weinberg equilibrium (heterozygote deficiency or excess). It is calculated for each polymorphic locus across all populations based on Wright (1978). Also, the significance of deviation genotypic frequencies from those expected under Hardy-Weinberg equilibrium was tested with Chi-square test per polymorphic locus over all populations.

(vii) Genetic distance (D): measures the gene differences between populations. It is computed between all pairs of populations based on Nei's (1978) unbiased genetic identity and genetic distance and these are used to cluster the populations by the unweighted pair group method with arithmetic averaging (UPGMA).

4. Results

4.1 Isozyme variation

A total of 17 alleles were observed from eight genetic loci for the three enzyme systems surveyed. The eight loci are as follows: Aat-1, Aat-2, Aat-3, Pgi-1, Pgi-2, Est-1, Est-2, and Est-3. Additional isozymes for AAT and EST were occasionally visualized in the gels, but were not included in the analyses because of their sporadic occurrence. PGI and AAT were interpreted as dimmers and EST was interpreted as monomer.

A polymorphic locus is defined here as one that has more than one allele at a locus. Based on this, polymorphism was detected in all populations for a minimum of three loci. All in all, the polymorphic loci revealed 14 different alleles are detected. Allelic variation among the eight loci was not partitioned equally (Table 2), three loci (Aat-2, Aat-3 and Est-1) are found to be monomorphic in all populations and two loci (Aat-1 and Pgi-1) were minimally variable with only two alleles per locus. An additional two loci (Est-2 and Est-3) were tri-allelic and Pgi-2 was tetraallelic. Average allele frequencies indicated that three out of the five polymorphic loci (Pgi-2, Est-2 and Est-3) were highly polymorphic, with allele frequencies $\leq$ 0.8 for the most common allele. Four populations (F8, A8, C10, H7 and L18) are monomorphic with alternative alleles for Aat-1, and from the remaining populations Aat-1^a is the highest frequency allele in B19 and O19 whereas Aat-1^b is the highest frequency allele in G8, V7, A17, J19, and D17. Pgi-1^b is present only in J19 and L18 but in low frequency. Pgi-2^a allele is found in highest frequency only in G8. C10 and B19 have Est-2^c as the highest frequency allele while in other populations Est-2^b is the highest frequency allele. Est-2^c is absent only in V7. A8 is the only population containing Est-3^c

as low frequency, unique allele. Only F8 and V7 have Est-3^a as the highest frequency allele.

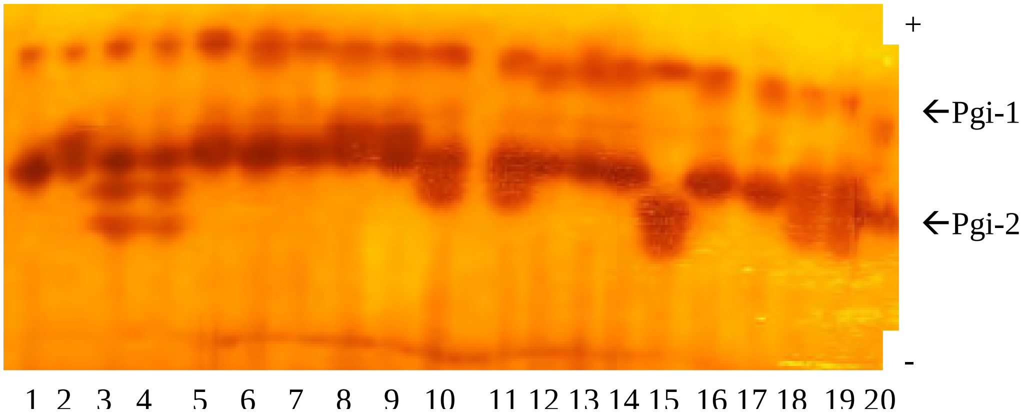

Figure 1. Photograph of starch gel showing the banding patterns of PGI.

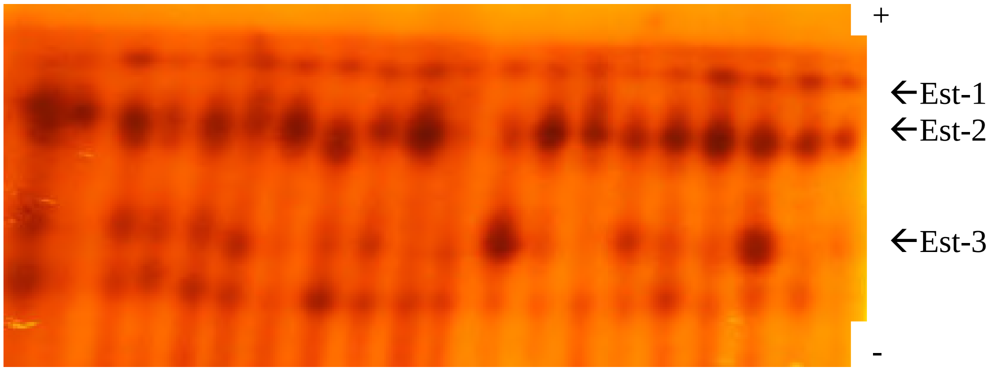

Figure 2. Photograph of starch gel showing the banding patterns of EST.

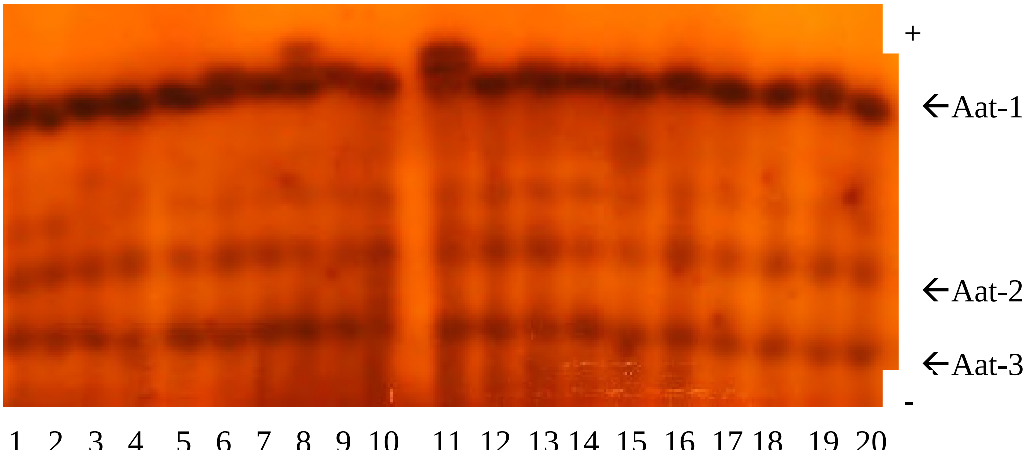

Figure 3. Photograph of starch gel showing the banding patterns of AAT.

4.2 Genetic diversity within populations

Average number of alleles per locus, percent of loci polymorphic, mean observed and expected proportion of loci heterozygous are calculated for each populations (Table 3). The mean number of alleles per locus ranged from 1.625 to 1.875, the lowest in population V7 and the highest in populations G8, A17, J19, D17 and O19. The percentage polymorphic loci ranged from 37.5-62.5%, the lowest in populations F8, A8, C10 and H7, and the highest in population J19. The mean gene diversity (H_{ep}) for the populations ranged from 0.1901 in population H7 to 0.2599 in population O19. When all populations are pooled as one (Table 4), there are 2.1250 alleles per locus (A_s) and the percentage of polymorphic loci (P_s) was 62.50%, the total observed heterozygosity ($\underline{H}_{os}$) and the total expected heterozygosity ($\underline{H}_{es}$) were 0.2698 and 0.2761, respectively. The average of mean gene diversities of all the populations ($\underline{H}_{ep}$) equals 0.218 using the formula $\underline{H}_{ep} = (1-F_{ST})H_{es}$, where H_{es} is the total genetic diversity and F_{ST} is the mean population differentiation (Nei, 1987).

Table 2. Mean allele frequencies of the twelve populations examined in this study.

Locus	Allele	Population											
		F8	**A8**	**C10**	**H7**	**G8**	**V7**	**A17**	**J19**	**D17**	**B19**	**L18**	**O19**
Aat-1	a				1.00	0.15	0.15	0.20	0.15	0.10	0.95	1.00	0.70
	b	1.00	1.00	1.00		0.85	0.85	0.80	0.85	0.90	0.05		0.30
Aat-2	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	b												
Aat-3	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	b												
Pgi-1	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.90	1.00	1.00	0.70	1.00
	b								0.10			0.30	
Pgi-2	a	0.25	0.10	0.25	0.20	0.40	0.15	0.10		0.15	0.20		0.15
	b	0.45	0.65	0.45	0.50	0.35	0.55	0.60	0.70	0.70	0.50	0.75	0.55
	c	0.20	0.25	0.25	0.15	0.20		0.25	0.25	0.05	0.30	0.20	0.25
	d	0.10		0.05	0.15	0.05	0.30	0.05	0.05	0.10		0.05	0.05
Est-1	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	b												
Est-2	a	0.30	0.10	0.05	0.10	0.25	0.25	0.25	0.05	0.15	0.10	0.10	0.25
	b	0.65	0.70	0.30	0.70	0.65	0.75	0.65	0.55	0.50	0.25	0.70	0.70
	c	0.05	0.20	0.65	0.20	0.10		0.10	0.40	0.35	0.65	0.20	0.05
Est-3	a	0.55	0.35	0.45	0.20	0.35	0.60	0.40	0.45	0.40	0.45	0.45	0.50
	b	0.45	0.60	0.55	0.80	0.65	0.40	0.60	0.55	0.60	0.55	0.55	0.50
	c		0.05										

Table 3. Summary of genetic variation within populations of noug including: percent polymorphic loci (P_p),average number of alleles per locus (A_p), mean observed (H_{op})and expected (H_{ep}) proportion of heterozygotes.

Population	**P_p**	**A_p**	**H_{op}**	**H_{ep}**
F8	37.50	1.750	0.2875	0.2191
A8	37.50	1.750	0.1875	0.1947
C10	37.50	1.750	0.3000	0.2171
H7	37.50	1.750	0.2375	0.1901
G8	50.00	1.875	0.3375	0.2487
V7	50.00	1.625	0.2750	0.2230
A17	50.00	1.875	0.2625	0.2461
J19	62.50	1.875	0.2500	0.2513
D17	50.00	1.875	0.3000	0.2289
B19	50.00	1.750	0.3000	0.2257
L18	50.00	1.750	0.2000	0.2329
O19	50.00	1.875	0.3000	0.2599

Table 4. Summary of genetic variation calculated pooling up all populations. P_s = Total percent polymorphic loci, A_s = total allele frequency, H_{os} = total observed proportion of heterozygetes, and H_{es} = total expected proportion of heterozygetes.

Locus	**P_s**	**A_s**	**H_{os}**	**H_{es}**
Aat-1	62.50	2.00	0.1333	0.4664
Aat-2		1.00	0.0000	0.0000
Aat-3		1.00	0.0000	0.0000
Pgi-1		2.00	0.0000	0.0647
Pgi-2		4.00	0.7583	0.6151
Est-1		1.00	0.0000	0.0000
Est-2		3.00	0.5167	0.5654
Est-3		3.00	0.7500	0.4968
Mean		2.1250	0.2698	0.2761
St. dev.		1.1260	0.3464	0.2821

4.3 Genetic diversity between populations

In this survey, the mean F_{ST} for all populations is 0.2117, and the average gene flow (Nm) between populations was 0.9312 individuals per generation (Table 5). The degree of differentiation (F_{ST}) of the individual loci ranged from 0.0 for Aat-2, Aat-3 and Est-1 to 0.6806 for Aat-1.

When all populations are pooled up, significant deviation from Hardy-Weinberg expectations was revealed for all polymorphic loci except Pgi-2 (Table 6). From these four loci that showed significant deviation three of them (Aat-1, Pgi-1, Est-2) are with positive F_{IS} indicating significant heterozygote deficiency.

4.4 Genetic distance and genetic identity

Genetic identity and genetic distance were determined for each pair of the twelve populations using the methods of Nei (1978) (Table 7). The distance between populations ranged from 0.0012 to 0.2346. The highest distance (0.2346) was between populations L18 from Gojam and population C10 from Welo. The smallest genetic distance (0.0012) was observed between the two Gojam populations: J19 and D17.

A dendrogram constructed based on Nei's (1978) unbiased genetic distance (Figure 4) defines two main clusters. The first main cluster is mainly composed of Gojam populations (B19, O19, L18) and one Welo population (H7). In the second main cluster, there are two subclusters, one consisting of mainly Gojam populations (D17, J19, A17) and one Welo population (A8) and the other subcluster consisting exclusively of Welo populations (V7, G8 and F8). In addition, C10 from the second main cluster is more genetically distant from the other populations.

Table 5. Summary of F_{ST} for all populations pooled as one, for Welo and Gojam populations pooled separately.

Locus	**All populations**		**Welo populations**		**Gojam populations**	
	F_{ST}	**Nm**	F_{ST}	**Nm**	F_{ST}	**Nm**
Aat-1	0.6806	0.1173	0.7496	0.0835	0.5762	0.1839
Aat-2	0.0000	****	0.0000	****	0.0000	****
Aat-3	0.0000	****	0.0000	****	0.0000	****
Pgi-1	0.2241	0.8654	0.0000	****	0.1964	1.0227
Pgi-2	0.0620	3.7815	0.0518	4.5786	0.0391	6.1382
Est-1	0.0000	****	0.0000	****	0.0000	****
Est-2	0.1379	1.5624	0.1431	1.4973	0.1232	1.7796
Est-3	0.0390	6.1676	0.0720	3.2218	0.0048	51.9706
Mean	0.2117	0.9312	0.1970	1.0192	0.1820	1.1235

4.5 Comparison of Gojam and Welo populations

When the Gojam and Welo populations are pooled separately, a total of 16 alleles is recorded in each region. Except Aat-1, both regions have the same highest frequency allele in all polymorphic loci (Table not shown). As it is shown in tables 8 and 9, the populations collected from Welo have A_s = 2.0, P_s = 50.0%, H_{os} = 0.2708 and H_{es} = 0.2570 while the Gojam populations resulted A_s = 2.0, P_s = 62.5%, H_{os} = 0.2687 and H_{es} = 0.2820. Regarding population differentiation (F_{ST}) the Gojam populations had F_{ST} = 0.1820 while in Welo F_{ST} = 0.1970 (Table 5). Using the formula $\underline{H}_{ep} = (1-F_{ST})H_{es}$, the averages of mean gene diversities ($\underline{H}_{ep}$) of Gojam and Welo populations are 0.2307 and 0.2064, respectively.

Table 6. Fixation index (F_{IS}) of all populations pooled together.

Allele \ Locus	Aat-1**	Aat-2	Aat-3	Pgi-1**	Pgi-2	Est-1	Est-2**	Est-3**
Allele A	0.7129	****	****	1.0000	-0.1940	****	-0.0716	-0.5137
Allele B	0.7129	****	****	1.0000	-0.3037	****	-0.0693	-0.5271
Allele C	****	****	****	****	-0.2435	****	0.3932	-0.0042
Allele D	****	****	****	****	-0.0860	****	****	****
Total	0.7129	****	****	1.0000	-0.2380	****	0.0824	-0.5161

**Chi-square test of the locus for the deviation from Hardy-Weinberg equilibrium is significant at P = 0.001

Table 7. Genetic identity (above diagonal) and genetic distance (below diagonal) determined for each pair of the twelve populations based on Nei's (1978) unbiased genetic identity and genetic distance.

pop ID	F8	A8	C10	H7	G8	V7	A17	J19	D17	B19	L18	O19
F8	****	0.9904	0.9615	0.8252	0.9942	0.9937	0.9929	0.9744	0.9834	0.8146	0.8131	0.9252
A8	0.0097	****	0.9716	0.8419	0.9859	0.9767	0.9974	0.9948	0.9953	0.8290	0.8315	0.9216
C10	0.0393	0.0288	****	0.8102	0.9619	0.9352	0.9594	0.9821	0.9846	0.8619	0.7909	0.8805
H7	0.1921	0.1721	0.2105	****	0.8802	0.8594	0.8934	0.8677	0.8637	0.9611	0.9738	0.9735
G8	0.0058	0.0142	0.0389	0.1276	****	0.9792	0.9944	0.9696	0.9820	0.8564	0.8444	0.9451
V7	0.0063	0.0236	0.0670	0.1516	0.0210	****	0.9885	0.9691	0.9794	0.8306	0.8545	0.9462
A17	0.0071	0.0026	0.0414	0.1127	0.0057	0.0116	****	0.9923	0.9938	0.8744	0.8825	0.9643
J19	0.0259	0.0052	0.0180	0.1419	0.0308	0.0314	0.0077	****	0.9988	0.8823	0.8773	0.9367
D17	0.0167	0.0047	0.0155	0.1465	0.0182	0.0208	0.0063	0.0012	****	0.8690	0.8524	0.9305
B19	0.2050	0.1876	0.1487	0.0397	0.1550	0.1856	0.1342	0.1252	0.1404	****	0.9505	0.9486
L18	0.2069	0.1845	0.2346	0.0266	0.1691	0.1572	0.1250	0.1309	0.1597	0.0508	****	0.9690
O19	0.0778	0.0817	0.1273	0.0268	0.0564	0.0553	0.0364	0.0654	0.0721	0.0527	0.0315	****

Table 8. Summary of genetic variability for Welo populations pooled together.

Locus	**P_S**	**A_s**	**H_{os}**	**H_{es}**
Aat-1	50.0	2.00	0.1000	0.3423
Aat-2		1.00	0.0000	0.0000
Aat-3		1.00	0.0000	0.0000
Pgi-1		1.00	0.0000	0.0000
Pgi-2		4.00	0.8667	0.6709
Est-1		1.00	0.0000	0.0000
Est-2		3.00	0.5500	0.5433
Est-3		3.00	0.6500	0.4999
Mean		2. 00	0.2708	0.2570
St. dev.		1.1952	0.3584	0.2888

Table 9. Summary of genetic variability for Gojam populations pooled together.

Locus	**P_s**	**A_s**	**H_{os}**	**H_{es}**
Aat-1	62.50	2.00	0.1667	0.5036
Aat-2		1.00	0.0000	0.0000
Aat-3		1.00	0.0000	0.0000
Pgi-1		2.00	0.0000	0.1255
Pgi-2		4.00	0.6500	0.5440
Est-1		1.00	0.0000	0.0000
Est-2		3.00	0.4833	0.5856
Est-3		2.00	0.8500	0.4973
Mean		2.00	0.2687	0.2820
St. dev.		1.069	0.3440	0.2724

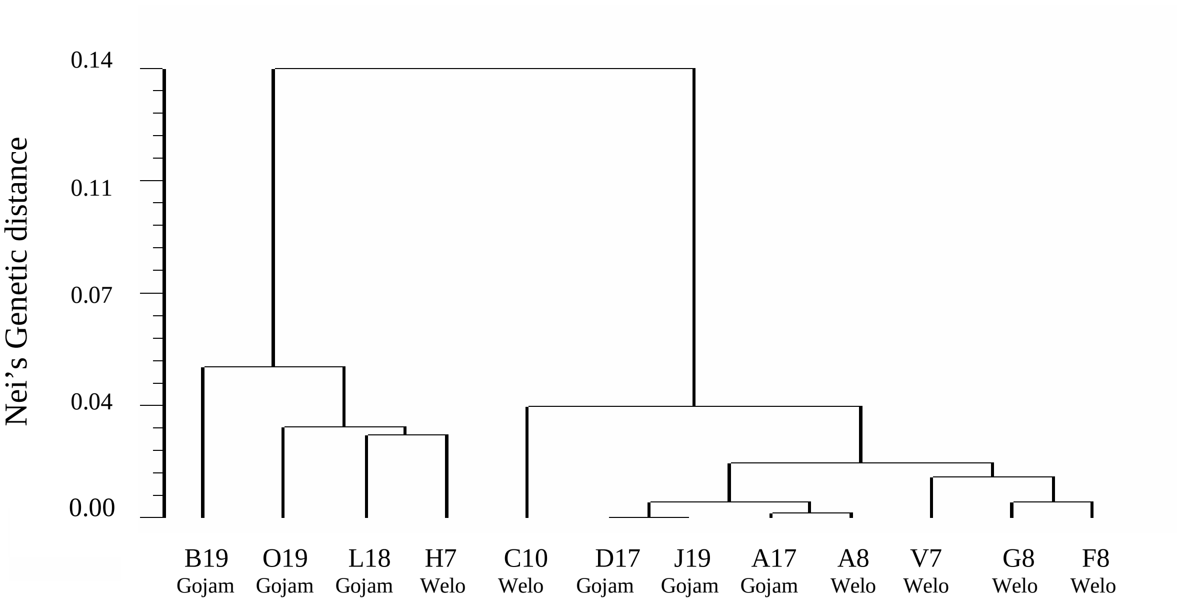

Figure 4. Dendrogram summarizing the relationship between the twelve populations. The dendrogram is calculated based on Nei's (1978) unbiased genetic identity and genetic distance.

5. Discussion

For the estimation of genetic variability within populations and genetic distance between populations a large number of individuals and large number of loci (enzyme systems) are required (Gottlieb, 1977; Murphy *et.al.*, 1996). In the present study, both number of loci and number of individuals studied are limited and thus values obtained from this study have limitations.

5.1 Isozyme variation

The number of isozymes for EST and PGI is the same as normally found in diploid plants (Wendel and Weeden, 1989; Kepart, 1990). Three isozymes were detected for AAT whereas most diploid plants have either two or four

(Wendel and Weeden, 1989; Kepart, 1990). However, three isozymes for AAT have been reported in *Helianthus annus* L. (Cronn *et.al.*, 1997) and *Coreopsis cyclocarpa* (Crawford and Bayer, 1981) which belong to the same family as *G. abyssinica*. In conspecific populations, the same allele is usually fixed at monomorphic genes and at polymorphic genes only the frequency of alleles differs (Gottlieb, 1977). In the present study high polymorphism of the polymorphic loci and absence of rare alleles (allele frequency < 0.05) roughly follows the expectations of outbreeding plants. If the number of plants that need to be examined from each population is small, it can lead to biased conclusions on whether particular low-frequency alleles are present in one population but lacking in another (Crawford, 1990). Thus, the small sample size used in this study could have introduced some biases.

5.2 Genetic diversity within populations

The highest polymorphism (P_p= 62.5%) was detected in population J19 from Gojam and the rest populations collected from Gojam showed P_p = 50.0%. But in Welo only two populations showed 50.0% polymorphism while the rest have P_p = 37.5%. This indicates the existence of high polymorphism within populations and that the populations from Gojam showed higher polymorphism than the populations collected from Welo. The highest and the lowest mean gene diversities observed were in O19 (H_{ep} = 0.2599) and in H7 (H_{ep} = 0.1901), respectively. Thus, O19 is the most genetically variable population while H7 is the least variable population.

Life history traits, especially, breeding system and life form had significant influences on genetic diversity and distribution (Hamrick, 1982). In the present study, levels of isozyme variation found in populations ($\underline{H}_{ep}$ = 0.218) are almost equivalent to the average value for 76 predominantly outcrossing plants (H_{ep} = 0.214[±0.034]) and greater than 62 annual plants (H_{ep} = 0.136[±0.025]) (Hamrick and Godt, 1996). However, the mean estimated heterozygosity is lower than 8 bee-pollinated plants (H_{ep} = 0.304[±0.127] (Hamrick and Godt, 1996). Based on the life form, higher than expected gene diversity is observed in *G. abyssinica*. However, the genetic diversity maintained by a species is a function not only of its life history traits but also depends heavily on the species ecological traits and evolutionary history (Hamrick and Godt, 1996).

Crops and their near wild relatives often grow sympatrically and the latter frequently occur as weeds in cultivated fields (Doebley, 1989). Because of their close proximity, the crops can gain genetic material from their wild relatives through the process of introgressive hybridization that introduces new diversity in crop populations (Jarvis *et.al.*, 2000). Kifle Dagne (1994b) reported a high level crossability of *G. abyssinica* with three wild species: *G. scabra* ssp *scabra*, *G. scabra* ssp *schimperi* and *G. villosa*, and their occurrence together especially with *G. scabra* ssp *schimperi.* Thus, it is very probable that introgressive hybridization takes place between *G. abyssinica* and its wild relatives and this could be the reason for the relatively higher level of intrapopulation variability observed in *G. abyssinica.*

5.3 Genetic diversity between populations

The mean genetic differentiation between populations over all loci (F_{ST}) was 0.2117, which indicates that the between-population component accounts for approximately 21.17% of the detected variation. Marked differences in the extent of differentiaion (F_{ST}) were shown between two loci: Aat-1 and Pgi-1. The level of differentiation was moderate for Pgi-2 and Est-2, and low for Est-3 while it is zero for the rest three monomorphic loci.

Population differentiation which is based here as differentiation of allozyme frequencies among populations can be measured either using Wright's F_{ST} or Nei's G_{ST} (1987). F_{ST} is equivalent to the coefficient of gene differentiation

(G_{ST}) and the F_{ST} value can be converted to G_{ST} and vice versa using the formulas $H_{es} = H_{ep}/ (1-F_{ST})$; $H_{es} = H_{ep} + D_{ST}$ and $G_{ST} = D_{ST}/H_{es}$, where H_{es} is total genetic diversity, H_{ep} is mean gene diversity, and D_{ST} is the average gene diversity between populations (Nei, 1973; Cole, 2003). The F_{ST} value of 0.2117 equals G_{ST} of 0.2119.

The level of isozyme variation found between populations (mean G_{ST} = 0.2119) is higher than the average value reported for other outcrossing plants (mean G_{ST} of 76 predominantly outcrossing plants was 0.118(±0.036) and mean G_{ST} of 15 outcrossing annual plants = 0.161)(Loveless and Hamrick, 1984). The reason for this higher value of G_{ST} could be because niger has an annual life cycle (mean G_{ST} of 62 plants =0.430[±0.157]), and pollination by bee (mean G_{ST} of 8 plants = 0.224 [±0.073]), which are factors associated with higher G_{ST} value. G_{ST} values of > 0.1 represent high among population variation (Chamberlain, 1998b; Lienert *et.al.*, 2002). Thus, high amount of genetic differentiation exists between the populations.

G. abyssinica is grown in Ethiopia on diverse environments. It is grown on heavy black clay water logged soils where there is adequate rainfall. On the other hand, *G. abyssinica* is also grown on shallow soil with low rainfall (Getinet Alemaw and Sharma, 1996). Accordingly, the higher G_{ST} value reflects adaptation to strong environmental dissimilarities or high level of genetic drift maintained by restricted gene flow between populations (Wuletaw Tadesse and Endashaw Bekele, 2001). In addition, domestic populations could have higher G_{ST} value if they are exposed to intense agronomic selection (Cronn *et.al.*, 1997).

Nonconfirmity to the prediction of Hardy-Weinberg equilibrium in a self-incompatible crop is expected because self-incompatiblity does not allow free intercourses. Thus, self-incompatibility results in an increase in the frequency of heterozygotes and reduction in the frequency of homozygotes relative to random mating population (Weaver and Hedrick, 1989; Murphy *et.al.*, 1996). However, 60 percent of the polymorphic loci from this study have shown significant heterozygote deficiency, which is opposite to what normally expected. The possible explanation for this could be that the land tenure character in the regions where small-scale farmers grow *G. abyssinica* in small-land holdings, thus, making the populations more subdivided and isolated from one another. The subdivision of a population into many breeding units results Wahlund's effect where the frequency of homozygotes tends to be higher than the Hardy-Weinberg proportion. Also, the isolation by distance of population subdivisions makes the effect of drift stronger and result heterozygous deficiency (Nei, 1987; Gillespie, 1998; Jarvis *et.al.*, 2000).

5.4 Genetic distance and genetic identity

High genetic distance (D> 0.2) is observed between populations F8 and D17 (D = 0.2050), F8 and L18 (D = 0.2069), H7 and C10 (D = 0.2105), C10 and L18 (0.2346) which indicated that they are divergent populations or lack gene flow between them. The highest genetic identity I = 0.9988 was observed between populations J19 and D17. The high mean identity for populations indicates close relationship due to recent divergence (Crawford, 1990).

The dendrogram have revealed some level of clustering between populations of the same region indicating the effect of more gene flow between populations of the same region. The close association of H7 and A8 with Gojam populations could be because they were introduced from Gojam to Welo, while the three Gojam populations forming the second main cluster with other five Welo populations could be probably because the three Gojam populations were introduced from Welo to Gojam.

Another possible reason could be because the populations are more related by other factors than geographical location. Similar environmental factors such as rainfall, soil character or similar selection by farmers for similar agronomic traits can affect certain populations from the two regions similarly than the other populations.

5.5 Comparison of Gojam and Welo populations

The percentage polymorphic loci (P_S = 62.5%) of populations collected from Gojam is higher than Welo populations (P_S = 50%). The number of alleles per locus (A_S=2.0) is the same in both regions. The average of mean gene diversities of Gojam populations ($\underline{H}_{ep}$ = 0.2307) is higher than that of Welo populations ($\underline{H}_{ep}$ = 0.2064). Considering that P and A are particularly sensitive to small sample size (Nei, 1987), the Gojam populations have more genetic variability than the Welo populations based on the mean genetic diversity. This is in line with morphological data (Genet, 1994 cited Getinet Alemaw and Sharma, 1996) where higher phenotypic diversity is observed in Gojam than in Welo.

Higher value of population differentiation (F_{ST}) is observed in Welo populations suggesting more genetic differentiation between Welo populations. However, the F_{ST} (G_{ST}) value is highly dependent on the value of mean genetic diversity (H_{ep}). When H_{ep} is small, G_{ST} may be large even if the absolute gene differentiation is small and vice versa (Nei, 1987; Gillespie, 1998). Thus, the higher F_{ST} value observed in Welo could be because the $\underline{H}_{ep}$ of Welo populations is lower than that of Gojam populations.

6. Conclusion and recommendations

The present results of isozyme analysis have revealed high genetic variability among and within populations of *G. abyssinica*. This genetic variability could be utilized for the breeding of *G. abyssinica* varieties for certain climatic and soil conditions, or widely adapted varieties that are drought tolerant, disease and pest resistant with high oil content and better oil quality.

The high amount of diversity revealed strengthens the results of early works on *G. abyssinica*. Gojam is suggested to be the probable center of diversity for *G. abyssinica* (Genet, 1994 cited in Getinet Alemaw and Sharma, 1996). While Mulatu Geleta (2001) suggested that South Welo could be the diversity center of *G. abyssinica*. In addition, these results indicate that allozyme markers have sufficient power to resolve variability among or within populations.

In order to make more reliable conclusion about the genetic variability of *G. abyssinica*, isozyme variation for *G. abyssinica* throughout Ethiopia has to be analyzed using large sample size and large number of enzyme systems. Modern molecular techniques such as Randomly Amplified DNA Polymorphism (RAPD), Restriction Fragment Length Polymorphism (RFLP) and Amplified Fragment Length Polymorphism (AFLP) have to be applied to construct genetic maps of this species and to tag agronomic traits for better oil quality, high yield and to determine the position of resistance genes to various disease causing organisms and other environmental factors such as drought.

Isozyme analysis is reasonably cheap in terms of time and expenses, which makes it desirable to predict patterns of variation for polygenic traits from those observed for isozyme loci. Thus, researches have to be conducted to check the correlation of the patterns of allozyme variation with the patterns of genetic variation found for other traits (e.g. morphological diversity) in *G. abyssinica*.

7. References

Adefris Tekelewold and Girma Mamo (2002). Response of noug [*Guizotia abyssinica* (L.f.) Cass.] to weather factors. *Eth. J. Nat. Res.* **4**(2):145-164.

Baagoe, J. (1974). The genus *Guizotia* (Compositae): a taxonomic revision. *Bot. Tidsskr.* **69**: 1-39.

Bayer, R.J. and Crawford, D.J. (1986). Allozyme divergence among five diploid species of *antennaria* (Asteraceae: Inuleae) and their allopolyploid derivatives. *Amer. J. Bot.* **73**(2): 287-296.

Carrera, A.D., Pizarro, G., Poverene, M., Feingold, S., Leon, A.J. and Berry, S.T. (2002). Variability among inbred lines and RFLP mapping of sunflower isozymes. *Genet. Mol. Biol.* **25**: 65-72.

Central Statistical Authority (2004). *Statistical Abstract: Ethiopia.* Addis Ababa., pp. 96.

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

Chamberlain, J.R. (1998b). Isozyme variation in *Calliandra calothyrsus* (leguminosae): its implications for species delimitations and conservation. *Amer. J. Bot.* **85**: 1-23.

Cole, C.T. (2003). Genetic variation in rare and common plants. *Annu. Rev. Ecol. Evol. Syst.* **34**: 213-237.

Crawford, D.J. (1990). *Plant Molecular Systematics.* John Wiley and Sons, U.S.A., pp. 65-104.

Crawford, D.J. and Bayer, R.J. (1981). Allozyme divergence in *Coreopsis cyclocarpa* (Compositae). *Systematic Botany* **6**(4): 373-379.

Cronn, R., Brothers, M., Klier, K., Bretting, P.K. and Wendel, J.F. (1997). Allozyme variation in domesticated annual sunflower and its wild relatives. *Theor. Appl. Genet.* **95**:532-545.

Doebley, J. (1989). Isozyme evidence and the evolution of crop plants. **In**: *Isozymes in Plant Biology*, pp. 165-192, (Soltis, D.E. and Soltis, P.S., eds). Chapman and Hall, London.

Frankel, O.H. (1974). Genetic conservation: our evolutionary responsibility. *Genetics* **78**: 53-65.

Getinet Alemaw and Adefris Tekelewold (1995). An agronomic and seed-quality evaluation of noug (*Guizotia abyssinica* Cass.) germplasm in Ethiopia. *Plant breeding.* **114**: 375-376.

Getinet Alemaw and Sharma, S.M. (1996). *Niger (G. abyssinica (L.f.) Cass.): Promoting the Conservation and use of Underutilized and Neglected Crops. 5.* International Plant Genetic Resource Institute, Gatersleben/International plant genetic resource institute, Rome.

Gillespie, J.H. (1998). *Population Genetics: A Concise Guide*. The John Hopkins University Press. London.

Gottlieb, L.D. (1977). Electrophoretic evidence and plant systematics. *Ann. Missouri Bot. Gard.* **64**:161-180.

Hamrick, J.L. (1982). Plant population genetics and evolution. *Amer. J. Bot.* **69**(10): 1685-1693.

Hamrick, J.L. (1989). Isozymes and the analysis of genetic structure in plant populations. **In**: *Isozymes in Plant Biology*, pp. 87-106, (Soltis, D.E. and Soltis, P.S., eds). Chapman and Hall, London.

Hamrick, J.L. and Godt, M.J.W. (1996). Effects of life history traits on genetic diversity in plant species. *Phil. Trans. R. Soc. Lond. B.* **351**: 1291-1298.

Hiremath, S.C. and Murthy, H.N. (1988). Domestication of niger (*Guizotia abyssinica*). *Euphytica* **37**:225-228.

Hiremath, S.C. and Murthy, H.N. (1992). Cytogenetical studies in *Guizotia* (Asteraceae). *Caryologia* **45**(1): 69-82.

Jarvis, D.I., Myer, L., Klemick, H., Guarino, L., Smale, M., Brown, A.H.D., Sadiki, M., Sthapit, B., and Hodgkin, T. (2000). *A Training Guide for in situ Conservation on-farm.* Version I. International Plant Genetic Resource Institute, Rome.

Kephart, S.R. (1990). Starch gel electrophoresis of plant isozymes: a comparative analysis of techniques. *Amer. J. Bot.* **77**(5): 693-712.

Kifle Dagne (1994a). Cytology, phylogeny and oil quality of *Guizotia* Cass. (Compositae). Ph.D. Dissertation, Addis Ababa University. pp. 1-35.

Kifle Dagne (1994b). Meiosis in interspecific hybrids and genomic interrelationships in *Guizotia* Cass. (Compositae). *Hereditas* **121**: 119-129.

Kifle Dagne (1995). Karyotypes, C-banding and nuclear numbers in *Guizotia* (Compositae). *Pl. Syst. Evol.* **195**: 121-135.

Kifle Dagne (2001a). Cytogenetics of new *Guizotia* Cass. (Compositae) interspecific hybrids pertaining to genomic and phlogenetic affinities. *Pl. Syst. Evol.* **230**: 1-11.

Kifle Dagne (2001b). Meiotic properties of induced autopolyploid *Guizotia abyssinica* (L.f.) Cass. *J.Genet. & Breed.* **55**: 11-16.

Kifle Dagne, Cheng, B. and Heneen, W.K. (2000). Number and sites of rDNA loci of *Guizotia abyssinica* (L.f.) Cass. as determined by fluorescence *in situ* hybridization. *Hereditas* **132**: 63-65.

Kifle Dagne and Heneen, W.K. (1992). The karyotype and nucleoli of *Guizotia abyssinica* (Compositae). *Hereditas* **117**:73-83.

Kifle Dagne and Jonsson (1997). Oil content and fatty acid composition of seeds of *Guizotia* Cass (Compositae). *J.Sci.Food Agri.* **73**: 274-278.

Kephart, S.R. (1990). Starch gel electrophoresis of plant isozymes: a comparative analysis of techniques. *Amer. J. Bot.* **77**(5): 693-712.

Linert, J., Fischer, M., Schneller, J. and Diemer, M. (2002). Isozyme variability of the wetland specialist *Swertia perennis* (Gentianaceae) in relation to habitat size, isolation, and plant fitness. *Amer. J. Bot.* **89**(5): 801-811.

Loveless, M.D. and Hamrick, J.L. (1984). Ecological determinants of genetic structure in plant populations. *Ann. Rev. Ecol. Syst.* **15**: 65-95.

Mulatu Geleta (2001). Ethnobotanical study of edible oil crops as a companion of *sorghum bicolor* L. Moench and biochemical genetic analysis of *in situ* and *ex situ* consereed *Guizotia abyssinica* (L.f.) Cass. germplasm from North Shewa and South Welo. M.Sc. thesis, Addis Ababa University.

Murphy, R.W., Sites, J.W., Buth, D.G. and Haufler, C.H. (1996). Proteins: isozyme electrophoresis. **In**: *Molecular Systematics*, pp. 51-121, (Hills, D.M., Moritz, C.and Mable. B.K., eds). Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts U.S.A.

Murthy, H.N., Hiremath, S.C. and Salimath, S.S. (1993). Origin, evolution and genome differentiation in *Guizotia abyssinica* and its wild species. *Thoer. Appl. Genet.* **87**: 587-592.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proc. Nat. Acad. Sci.* USA. **70**(12): 3321-3323.

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

Nei, M. (1987). *Molecular Evolutionary Genetics*. Columbia university press, New York, pp. 149-208.

Nei, M. and Roychoudhury, A.K. (1974). Sampling variance of heterozygosity and genetic distance. *Genetics* **82**: 543-545.

Parker, P.G., Snow, A.A., Schug, M.D., Booton, G.C. and Fuerst, P.A. (1998). What molecules can tell us about populations: choosing and using a molecular marker. *Ecology* **79**(2): 361-382.

Quinn, J. and Myers, R.L. (2002). Nigerseed: speciality grain opportunity for midwestern US. **In**: *Trends in New Crops and New Uses*, pp. 174-182, (Janick, J. and Whipkey, A. eds.). ASHS Press, Alexandria, VA.

Ramachandran, T.K. and Menon, P.M. (1979). Pollination mechanism and inbreeding depression in niger (*Guizotia abyssinica* Cass.). *Madras agric. J.* **66**(7): 449-464.

Ramadan, M.F. and Morsel, J.T. (2002). Proximate neutral lipid composition of niger (*Guizotia abyssinica* Cass.) seed. *Czech J.Food Sci.* **20**(3): 98-104.

Riley, K.W. and Belayneh, H. (1989). Niger. **In**: *Oil Crops of the World*, pp. 394-403, (Robbelen, G., Downey, R.K. and Ashri, A., eds.). McGraw Hill publishing company, New York.

Seegeler, C.J.P. (1983). *Oil plants in Ethiopia, their Taxonomy and Agricultural Significance.* Center for Agricultural Publishing and Documentation. Wageningen., pp. 122-146.

Sileshi Nemomissa, Endashaw Bekele and Kifle Dagne (1999). Self-incompatibility system in the Ethiopian populations of *Guizotia abyssinica* (L.f.) Cass. (niger). *SINET: Ethiop. J. Sci.* **22**(1): 67-88.

Weaver, R.F. and Hedrick, P.W. (1989). *Genetics* (2nd ed.). Wm. C. Brown Publishers.

Weeden, N.F. and Wendel, J.F. (1990). Isozyme analysis of plant mating systems. **In**: *Isozymes in Plant Biology*, pp. 46-73, (Soltis, D.E. and Soltis, P.S., eds). Chapman and Hall, London.

Wendel, J.F. and Weeden, N.F. (1990). Visualization and interpretation of plant isozymes. **In**: *Isozymes in Plant Biology*, pp. 5-46, (Soltis, D.E. and Soltis, P.S., eds). Chapman and Hall, London.

Wright, S. (1978). *Variability within and among Natural Populations.* Vol. 4. The Univ. of Chicago Press, Chicago.

Wuletaw Tadesse and Endashaw Bekele (2001). Isozyme variability of grasspea (*Lathyrus sativus* L.) in Ethiopia. *Lathyrus Lathyrism Newsletter* **2**: 43-46.

Yeh, F.C., Yang, R-C. and Boyle, T. (1999). POPGENE VERSION 1.31: Microsoft window –based freeware for population genetic analysis. University of Alberta.

Yohannes Petros (1983). Anatomical studies of oil bearing species of Compositae: *Vernonia galamensis* (Cass.) Less. and *Guizotia abyssinica* (L.f.) Cass. M.Sc. thesis, Addis Ababa University.

DECLARATION

I, the undersigned, declare that this thesis is my own work and it has not been presented in other Universities, Collages or Institutions, seeking for similar degree or other purpose.

MUHAMMED AHMED	28th of July, 2006	____________________
NAME	DATE OF SUBMISSION	SIGNATURE