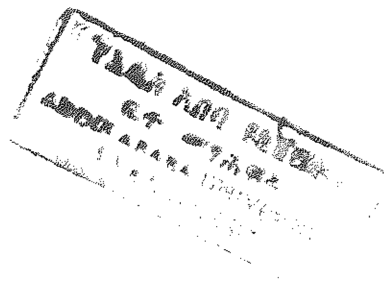
ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

Isozyme assessment of the relationships between *Guizotia abyssinica* (L.f) Cass. and its close wild relatives

By

Leggesse Taddesse



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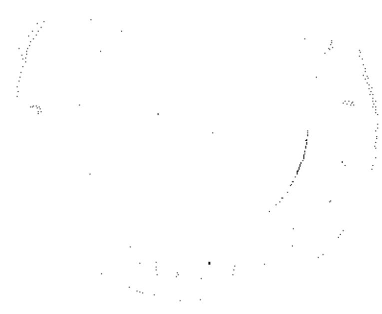

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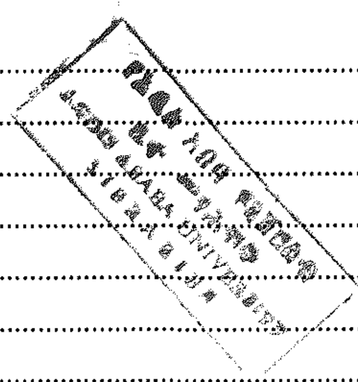

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Abstract

In this study, 13 populations, representing six species/taxa of *Guizotia* were compared using isozyme markers. The three enzyme systems, aspartate aminotransferase (AAT, E.C. 2.6.1.1), acid phosphatase (ACP, E.C. 3.1.3.2) and phosphoglucose isomerase (PGI, E.C. 5.3.1.9) were used to study the genetic variation of the cultivated *G. abyssinica* and five related wild taxa /species belonging to *Guizotia*. Eeven if, most of these taxa share common morphological and cytological features, the genetic bases of differences distinguishing them have not been determined. The electrophoretic study was initiated to assess the degree of divergences or genetic identities among these closely related groups of taxa with the possible identification of progenitor for the cultivated species, *G. abyssinica*. All the taxa/speices were analyzed, using horizontal starch gel electrophoresis. We found three AAT, two ACP and two PGI isozyme zones in all species /taxa except *G. arborescens* (has four PGI isozyme zones) in the studied materials and hence a total of eight loci were assessed. Since all species/taxa are assumed to be outcrossing, the genetic diversity within population is greater than among populations. A high level of genetic variation and low level of genetic differentiation among populations were found in all species /taxa except *G. arborescens*. Chelelu is recently identified population and show high genetic diversity than other studied material probably due to its adjacent growth with widespread *G. scabra* ssp *schimperi* and other related species may be favoured for its variability through introgression or due to its unique history. *G. abyssinica* and Chelelu showed low genetic distance than the genetic distance of *G. abyssininia* and *G. scabra* ssp *schimperi* at genes specifying the three enzyme systems. The idea of progenitor-derivative species pairs between *G. abysinica* and Chelelu is supported by the high genetic identity values(I = 0.9305). Furthermore, the putative derivative *G. abysinica* has similar allele with the possible progenitor Chelelu population. Most alleles which were found in *G. abysinica* are also found in Chelelu. The allozyme diversity indicated that Chelelu is the most probable proginetors of *G. abysinica*. Likewise, the isozyme study showed that there were close relationships among *G. abyssynica*, *G.scabra* ssp *schimperi* and Chelelu population, and between *G. scabra* ssp *scabra and G. villosa* in support of the karyotype and pollen fertility studies in the genus *Giuzotia*. The genetic marker used in this study also indicated that *G. arborescens* was the most divergent taxa among the studied materials which is in line with the cytological and morphlogical studies

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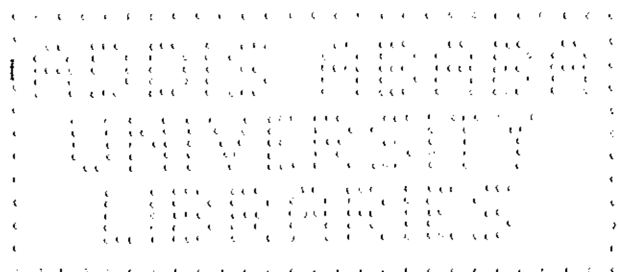
1. Introduction

Guizotia abyssinica (L.f.) Cass., (commonly called Noog or Niger) belongs to the family Compositae, tribe Heliantheae sub tribe Corepsidinae and the genus *Guizotia*. According to Baagoe (1974), the genus comprises six species namely: *G. abyssinica* (L.f.) Cass., *G. arborescens* I. Friis, *G. scabra* (Vis) Chov., *G. reptans* Hutch., *G. villosa* Sch.Bip., and *G. zavattarii* Lanza. As Kifle Dagne (1994) pointed out, there are possibly more *Guizotia* species, for example Ketcha and Chelelu populations for which the taxonomic position is not yet determined. Most of *Guizotia* species are native to Ethiopia and contain annual and perennial forms. *G. abyssinica* is the only cultivated form of the genus. It is believed that the cultivated crop originated through disruptive selection from *G. scabra* ssp *schimperi*, a common weed, in Ethiopia known as" Mech" (Baagoe, 1974; Hirmath and Murthy, 1988). Low oil content and shattering favoured by natural selection for the wild form (*G. scabra* ssp *schimperi)* whereas high oil content and low or no shattering favoured by artificial selection for the cultivated species (*G. abyssinica*).

The genus *Guizotia* occurs mainly in the tropical Eastern Africa with the highest species concentration in Ethiopia (Baagoe, 1974). *G. abyssinica* is cultivated in Ethiopia and some other Eastern part of African countries and Indian subcontinent. *G. scabra* ssp *scabra* is widely distributed in Eastern part of Africa. *G. scabra* ssp *schimperi* occurs in the Ethiopian highlands (Baagoe, 1974). *G. villosa* and *G. arborescens* are endemic to the northern part of the Ethiopian highlands. *G. reptans* is endemic to Mount Kenya and thus the only taxon of *Guizotia* not recorded so far from Ethiopia. From this point of view, it is evident that Ethiopia is the centre of genetic diversity for the genus and is probably the centre of origin (Kifle Dagne, 1994).

It is believed that *G. abyssinica* was domesticated in Ethiopia and reached India probably through trade routes (Hiremath and Murthy, 1988). Baagoe (1974) and Hiremath *et al.* (1992) suggested that Noog was derived from *G. scabra* ssp *schimperi* and acquired specific traits through selection under cultivation. This is also supported by Kifle Dagne (1994, 2001a). Cytological studies showed that all *Guizotia* taxa have 2n=30 chromosomes, indicating that speciation within the genus did not involve changes in the chromosome number (Hiremath and Murthy, 1992; Kifle Dagne, 1994).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The chromosome morphology of Chelelu was more related to *G. abyssinica* and *G. scabra* ssp *schimperi* than to other taxa in the genus (Kifle Dagne, 1994). This population could be suggested as a possible ancestor from which *G. scabra* ssp *schimperi* could be derived. *G. abyssinica is* more closely related to Chelelu and *G. scabra* ssp *schimperi* (Kifle Dagne, 1994, 2001a). Though *G. scabra* ssp *scabra* and *G. villosa* have some resemblance with *G. abyssinica,* they differ from the latter in terms of morphology and habit (Kifle Dagne, 1994, 2001a). According to Baagoe (1974) the evolutionary trends and phylogenetic relationships with in the genus indicate that *G. scabra* ssp *scabra* has the highest correlation of primitive characters (e.g. leave sesile), and *G. reptans* and *G. arborescens* have the highest correlation of advanced characters (e.g. petiolate leave), while the other taxa are approximately intermediate.

Baagoe (1974) and Hiremath *et al.* (1992) speculated that *G. scabra ssp schimperi* was a possible progenitor of *G. villosa.* However, the karyotype and chromosome pairing in hybrids indicated that *G. villosa* has more affinity to *G. scabra* ssp *scabra* than to *G. scabra* ssp *schimperi* (Kifle Dagne, 1994). The possibility of Chelelu population to be the possible progenitor of *G. abyssinica* in addition to *G. scabra* ssp *schimperi* and the question of considering *G. scabra* ssp *scabra* or *G. scabra* ssp *schimperi* as progenitor of *G. villosa* have not been resolved, and hence need further investigation with molecular markers.

From interspecific hybridisation point of view, *G. scabra* ssp *scabra* is more related to *G. villosa* than to *G. scabra* ssp *schimperi* (Kifle Dagne, 1994). Morphologically again, *G. scabra* ssp *schimperi* is more similar to *G. abyssinica* than to *G. scabra* ssp *scabra* which is characterized by its perennial habit, stiff leaves and higher number of florets (Baagoe 1974; Kifle Dagne, 1994; Getinet and Sharma, 1996).

From the chromosome pairing data, Murthy *et al. (*1993) suggested that *G. abyssinica* and *G. scabra* ssp *schimperi* are conspecific members of the same species for they cross easily, their F_1 meiosis is nearly complete and its F_1 are highly fertile. *Guizotia abyssinica* is more crossable with *G. scabra* ssp *schimperi* (41%) than with both *G. scabra* ssp *scabra* and *G. villosa* (20%). Likewise, *G. scabra* ssp *schimperi* is more crossable with both wild taxa *G. scabra* ssp *scabra* (62%) and *G. villosa* (46%). However, *G. scabra* ssp *scabra* is the most crossable with *G. villosa* (82%) than with *G. scabra* ssp *schimperi* (62%) (Kifle Dagne,

1994, 2001a). This is another indication of uncertainty with the taxonomic position of *G. scabra* ssp *schimperi.*

G. abyssinica is grown as an oil crop mainly in Ethiopia and India (Getinet and Sharma, 1996; Kifle Dagne, 1997, 2001a). The seeds are important sources of oil of nutritional, industrial and pharmaceutical importance. The oil quality of the Noog is not affected by the gene transfer from wild types to the cultivated ones that provide desirables features for human consumption since there is little difference in the oil composition between wild forms and Noog (Kifle Dagne and Johnson, 1997). The self-incompatibility and low yield in both cultivated and wild forms of *Guizotia* make interspecific hybridisation essential in the improvement programs of the crop.

The genus is, thus, economically important that 50-60% of the edible oils in Ethiopia comes from Noog. In India, it accounts for about 2% of the total oil seed produced (Riley and Belayneh, 1989; Kifle Dagne and Johnson, 1997). Besides this, it is used in the manufacture of soap and paints or lubricant. The protein rich meal after oil extraction is used as a feed, manure or fuel. In some localities wild *Guizotia* is used for human consumption and medicinal purposes (Fujimoto, *et al.,* 1990; Kifle Dagne and Johnson, 1997).

To determine the taxonomic and evolutionary relationships among these closely related taxa, biochemical and molecular studies are necessary in addition to cytological and morphological studies. The biochemical (isozyme) variation between the cultigen and its progenitors may become minimal because usually crops and its wild relatives lack genetics barrier to hybridization. Cultivated crops and their progenitors share a number of isozyme alleles either through introgression or inheritance from their common ancestor (Doebley, 1989). Generally, Ethiopian oil crops are well adapted to a wide range of environmental conditions and agricultural practices due to a high potential of genetic variability.

Particularly, *G. abyssinica,* to date shows a high demand of domestic and foreign market. Utilization of all possible techniques of crops improvement to increase the yield contributes to the economic growth of the country in general for food security in particular (Ellstrand *et al.*, 1999; Kandel and Lake, 2002; Quinn and Myers, 2002; Mulatu Geleta *et al.*, 2002).

should contain a subset of the alleles present in the progenitor. As a result of this, most crops show a reduction in levels of polymorphism as compared to their presumed progenitors (Doebley, 1989; Wiley and Sons, 1990).

In the evolutionary context domesticated plants represent lineages that diverge from their progenitors not more than a few thousand generations ago (Ellstrand *et al.*, 1999). Generally, whether a particular wild form is genetically similar enough to a crop to be considered a potential progenitor, it can be judge using Nei's (1972) measure of genetic identity (I). Genetic Identity between populations of a crop and those of its supposed progenitor should be with in the range of variation among populations of the progenitor (Doebley, 1989; Wiley and Sons, 1990; Murthy *et al.*, 1996).

2.1.2. Isozymic and morphological variation in crops and their wild relatives

Wide morphological differences may exist between the nearest wild relatives and cultivated crops. Conversely, wild species within a genus containing cultigens may be morphologically so similar that only an expert can readily distinguished them (Gottlieb, 1984: cited in Wiley and Sons, 1990; Doebley, 1989). When isozyme, rather than morphological variation, is considered, cultigens typically show close isozymic similarity to their progenitor species, and varieties within a single crop species also show small isozymic differences (Doebley, 1989). However, in many cases morphological intermediacy and molecular confirmation go hand in hand (Ellstrand *et al.*, 1999).

2.1.3. Introgression between crops and their wild relatives

Crops and their wild relatives often grow sympatricaly, and the latter frequently occur as weeds in cultivated fields that create a new opportunity for introgression (Agrawal, 1998; Doebley, 1989). Crops and their wild relatives often lack genetic barriers to hybridization, and naturally occurring hybrids are frequently reported (Ellstrand *et al.*, 1999)

On the other hand, the ability to detect isozyme alleles among crops and their wild relatives is limited by the fact that a crop and its progenitor possess the same assemblage of isozyme alleles. Therefore, the shared presence of an allele may indicate either introgression or joint inheritance from their common ancestor (Doebley, 1989). For instance, Zamir *et al.* (1984: cited in Doebley, 1989) examined isozyme variation in *Citrullus lanatus* (watermelon) and sympatric wild relatives, *C. colocynthis*. These species are fixed for distinct alleles at nine isozyme loci suggesting that there was considerable introgression of the wild species genes into the genetic background of the watermelon.

At the genome scale, the probability for crop gene transfer depends on the level of genetic and structural homology between the genome of crops and wild plants (Agrawal, 1998). More frequent introgression is expected when crops and their wild relatives share high level of homology (Agrawal, 1998; Jenczewski *et al.,* 2003). Generally, the spontaneous introgression of wild alleles into crop, or landraces serves as a substrate for crop improvement.

2.1.4. Origin of weed relative of crops

A near relative of a crop that may grow exclusively in disturbed habitats such as lakeshores and stream sides that under go frequent or seasonal natural disturbance is referred to as wild. On the other hand, a relative of a crop that may grow exclusively in human disturbed habitats especially agricultural fields is termed as weedy (Jenczewski *et al.,* 2003). The weed originated as the hybrid derivative of the crop and wild. Crop to weed gene flow has been considered in the evaluation of enhanced weediness in wild relatives of most important crops (Ellstrand *et al.,* 1999). Otherwise, weeds may have originated through the modification of wild species by natural selection in the absence of significant progressive hybridization (Doebley, 1989). Each of these modes of degree of isozymic similarity between the wild, weedy and the crops is usually considered in the account of crop evolution.

2.1.5. Loss of genetic variation in crops

Even if domestication results in loss of genetic variation over time, there are competing forces such as introgression of new variation from wild relatives, selection against loss of fitness due to inbreeding, and trading of cultivars among farmers over considerable geographical distances (Doebley, 1989). Bottlenecks during domestication have caused a reduction in genetic variation in crops and this can be addressed with isozymic data (Doebley, 1989; Murthy *et al.*, 1996; Cole, 2003). There are various measures of genetic diversity for crops and their wild relative, which in most cases represents their probable progenitor. These are total heterozygosity, mean expected heterozygosity, proportion of polymorphic loci per population/taxon, and alleles per locus per taxon (Cole, 2003). Since there are a number of reports that indicate the loss of genetic variation in crops as compared to its wild relatives, the wild progenitors represent a pool of new genetic variation for crop improvement programme (Ellstrand *et al.,* 1999).

2.1.6. Effect of domestication on genetic variation

The extents of isozyme variation in a crop species tend to be localized within landraces and cultivars or distributed more equally among them thus providing a picture of the genetic structure of crops (Doebley, 1989). This structure may be compared to that of its progenitor to determine whether domestication has altered it or not. If any change has occurred, it might be towards either greater concentration of variation within groups or greater dispersion of variation among groups depending on the operative forces (Doebley, 1989; Cole, 2003). Strong artificial selection, genetic bottlenecks, mode of reproduction of the crops (selfing vs. out crossing), exchanging practices among farmers and introgression from wild forms could all come into play (Doebley, 1989).

2.1.7. Evolutionary consequences of gene flow

A small amount of gene flow is capable of counteracting the other evolutionary forces of mutation, drift and selection. As Ellstrand *et al.* (1999) point out that the magnitude of gene flow among natural plant populations is varying among species, population, and individuals. However, there is no considerable genetic variation difference among population of rare and common species even if the level of gene flow affected by the status of the species (higher in common species and lower in rare species) (Cole, 2003). The best-known evolutionary consequence of gene flow is its tendency to homogenise population structure. The condition of homogenization will vary depending on the nature of immigrant alleles (Doebley, 1989; Ellstrand *et al.*, 1999). The immigrant alleles could be neutral (prevent interpopulation differentiation), detrimental (reduce local fitness and ultimately cause extinction) or beneficial (the alleles more-or-less fixed in the crop and absent from the wild population). This is the evolution of weediness and the likelyhoods of extinction of wild relatives due to outbreeding and detrimental gene flow are the possible adverse effect of gene flow between crop and its wild relatives (Ellstrand *et al.*, 1999).

2.2. Oil crops in Ethiopia

Ethiopia is one of the centres of world genetic diversity that contributes to modern commercial agricultural and the development of high yield varieties (Teshome, 1996: cited in Mulatu Geleta *et al.*, 2002). Among the different varieties of crops species, the oil plant species contribute oil production in the country. Even if the country has around 328 species of oil plant species, only 15 species are cultivated and economically important (Mulatu Geleta *et al.*, 2002). Noog, sesame, linseed, sunflower, safflower, Ethiopian mustard and caster are some of the economically important oil crops in Ethiopia. Based on their ecology, oil crops could be grouped into highland oil crops (e.g. noog, linseed) and lowland oil crops (e.g. ground nut, safflower) (Weyessa, 1987: cited in Mulatu Geleta *et al.*, 2002). One of the highland dominant oil crops in Ethiopia is *Guizotia abyssinica* commonly called niger or Noog (Baagoe, 1974; Kifle Dagne, 1994).

Generally, the Ethiopian annual oil crops are well adapted to a wide range of environmental conditions and agricultural practices because of their genetic variability (Getinet and Sharma, 1996). For their simple extraction process, high storability and attraction for export, the improvement programme should give due attention to oil crops within the country as well as a broad approach particularly for Noog (Quinn and Myers, 2002).

2.3. *Guizotia.* Cass.

2.3.1. Taxonomy

According to Baagoe (1974) the genus belongs to the family Compositae, tribe Heliantheae and contains 6 species. She reduced the status of two species, *Guizotia scabra* and *G. schimperi* into the status of sub species and merged them together as *G. scabra* ssp *scabra* and *G. scabra* ssp *schimperi,* respectively. Recently, the genus comprises *G. abyssinica* (L.f) Cass. *G. arborescens* I. Friis, *G. scabra* (vis.) choir.ssp *scabra, G. scabra* (vis.) Chiov.ssp *schimperi* (sch.Bip Baagoe, *G. reptans* Hutch., *G. villosa* Sch.Bip., and *G. zavattarii* Lanza. In addition, two new populations called Chelelu and Ketcha (both named after the localities from where they were collected in Ethiopia) have been identified by Kifle Dagne (1994). Among these taxa, *G. abyssinica, G. scabra* ssp *scabra, G. scabra* ssp *schimperi, G. villosa* and Chelelu population are closely related and the first three contribute for unsettled taxonomic issues of *Guizotia.*

The recent study of karyotype and morphology of *G. scabra* ssp *scabra* and *G. scabra* ssp *schimperi* provides a significant difference between them, suggesting that their taxonomic status should be revised (Hiremath and Murthy 1992; Kifle Dagne, 1994, 1995, 2001a). The other controversial issue lies on the taxonomical category of *G. abyssinica* and *G. scabra* ssp *schimperi* (Murthy *et al.,* 1993). *G. abyssinica* and *G. scabra* ssp *schimperi* are similar in morphology, karyotype and produce fertile hybrids with 95% of pollen fertility and percentage form 15 bivalents that make them close to each other than *G. scabra* ssp *scabra* (Kifle Dagne, 1994, 1995). Murthy *et al.* (1993) proposed that they have to be merged into a single species.

2.3.2. Morphological study of the genus

Most related taxon in the genus has greater similarity in their morphology and anatomy (Baagoe, 1974). *G. scabra* ssp *schimperi* and *G. abyssinica* are more similar to each other in terms of their annual habit, ovate outer phyllaries and lower number of floret than to *G. scabra* ssp *scabra*. According to Baagoe (1974), *G. scabra* ssp *scabra* shows primitive characters (e.g. caudex perennial habit, sessile leaves, solitary heads) and *G. reptans* and *G. arborescens* show advanced morphological features (e.g., petiolated leaves, few floret), while the other species are intermediate in their morphological status. However, each taxon within the genus can be distinguishable from one another by their growth patterns and diagnostic morphological features (Table 1).

Table 1. Diagnostic morphological features of *Guizotia* species/taxa.

No.	Taxon	Diagnostic features
1	*G. abyssinica*	Annual, ovate outer phyllaries and large size of achene.
2	*G. scabra* ssp *schimperi*	Annual, lower number of florets and ovate outer phyllaries and small achenes
3	*G. scabra* ssp *scabra*	Perennial, stiff leaves, higher number of florets and lanceolate outer phyllaries.)
4	*G. villosa*	Annual herb, small flower heads and long linear outer phyllaries
5	Chelelu	Perennial, Rhizomatous like vegetative propagation in addition to seed propagation, high number of ray florets, colour and size of achene
6	*G. arborescens*	Woody (perennial) shrubby habit, petiolated leaves with two lateral nerves
7	*G. reptans*	Creeping perennial, large achene size
8	*G. zavattarii*	perennial,pandutate,lanceorate leaves outer phyllaries and two strong side nerves

Source: Modified from Kifle Dagne, 1994; Baagoe, 1974.

2.3.3. Geographical distribution of the genus

Guizotia is native to tropical Africa (Baagoe, 1974). Most of the taxa occur in East Africa (e.g. ssp *scabra*) with the highest concentration of species in Ethiopia (5 out of 6). Niger is cultivated in Ethiopia and India. *G. scabra* ssp *schimperi* is native to Ethiopian highlands and *G. villosa* and *G. arborescens* are endemic to the northern highlands and south west of Ethiopia, respectively (Baagoe, 1974; Kifle Dagne, 1994). From the species concentration of this genus, it is evident that Ethiopia is the centre of genetic diversity and probably origin of the genus *Guizotia.* This is because the only taxon of *Guizotia* that is not recorded so far from Ethiopia is *G. reptans* (endemic to mountain Kenya) (Baagoe, 1974; Kifle Dagne, 1994; Getinet and Sharma, 1996).

2.3.4. Domestication in the genus

Guizotia abyssinica is the only taxon cultivated and consequently economically important oil seed crops in the genus (Baagoe, 1974; Riley and Belayneh, 1989; Kifle Dagne, 1994). Even if Niger has been cultivated in Ethiopia and India, there is a strong belief that this crop was first domesticated in Ethiopia and reached India through trade routes (Baagoe, 1974; Hiremath and Murthy, 1992; Kifle Dagne, 1994; Quinn and Myers, 2002).

According to Baagoe (1974), since *G. abyssinica* is derived from G. *scabra* ssp *schimperi* and acquired its specific characters through selection under cultivation, the lack of known wild type in India supports the domestication of Niger in Ethiopia. Furthermore, the cytological and morphological studies of the closely related taxa of *Guizotia* strengthen that *G. abyssinica* is derived from *G. scabra* ssp *schimperi* and hence domesticated in Ethiopia (Baagoe, 1974; Hiremath and Murthy, 1988; Kifle Dagne, 1994).

2.3.5. Cytology and karyotype of the genus

All the known species and the two new populations of the genus are diploid with 2n = 30 chromosomes (Hiremath and Murthy, 1992; Kifle Dagne 1994, 1995). This indicates that speciation within the genus did not involve change in chromosome number. However, individual chromosomes show considerable variation in length of arms, centrometric position, type of chromosome size that occurred in the course of speciation (Hiremath and Murthy, 1992; Kifle Dagne, 1994; Getinet and Sharma, 1996; Kifle Dagne *et al.,* 2000)

In addition to the normal chromosome complement, B-chromosomes have been found in *G. scabra* ssp *scabra* but is commonly considered as parasitic or selfish that has no known significant role in the genus (Hiremath and Murthy, 1986; Kifle Dagne, 1994).

The karyotype of *G. villosa, G. abyssinica* and *G. scabra* ssp *schimperi* are symmetrical, and they are consisting of median (m), submedian (sm) type of chromosomes (Hiremath and Murthy, 1992), thus these three taxa appeared to be primitive. However, Kifle Dagne (1995) found that the karyotype of *G. villosa* is strongly asymmetrical than the karyotype of the rest of studied *Guizotia* taxa because this species contain sm and sub terminal (st) chromosomal type predominantly.

As Kifle Dagne (1995) pointed out, the study of chromosome morphology showed that *G. villosa* could have evolved from *G. scabra* ssp *scabra* rather than suggested by Hiremath and Murthy (1992) (in which it was assumed to have evolved from *G. scabra* ssp *schimperi*). The karyotype of *G. abyssinica* and *G. scabra* ssp *schimperi* is similar but not identical (Kifle Dagne and Heneen, 1992; Kifle Dagne, 1995).

Based on chromosome asymmetry and its centrometric position, Kifle Dagne (1995) put the taxa of *Guizotia* into three groups. Groups one comprises *G. abyssinica, G. scabra* ssp *schimperi* and Chelelu population in which they have m type of chromosomes and 6 to 8 satellite chromosomes in common. In this group, Chelelu seems to be more related to *G. abyssinica and G. scabra* ssp *schimperi* than the rest of the taxa, and hence could be possible ancestor of either of the two. The second group contains *G. zavattarii* and *G. arborescens* having relatively large chromosomes and more asymmetrical in their karyotypes. Moreover,

the study of karyotype in this group shows that they are distantly related to the taxa, which are found in the first group.

The third group still is more asymmetrical than the rest taxa of *Guizotia.* This group contains *G. villosa, G. scabra* ssp *scabra,* and Ketcha population of which *G. villosa* has the most asymmetrical karyotype, with 26 of the chromosomes having telomeric C- banding on the long arm. In this group, the karyotype study shows that *G. scabra* ssp *scabra* to be more related to *G. villosa* than to *G. scabra* ssp *schimperi* in line with the findings of Kifle Dagne (1994). Thus, the chromosome morphology and karyotype showed the extent of closeness of *Guizotia* species in terms of their evolutionary relationship within the genus.

2.3.6. Interspecific hybrids within the genus

Interspecific crosses between most species/population within the genus *Guizotia* could produce normal seeds in both directions. Kifle Dagne (1994, 2001a) pointed out that there are different degrees of affinities between the different combinations of *Guizotia* taxa. Among the different crosses, the highest genomic affinity was observed between *G. scarbra* ssp *schimperi* and Chelelu (99.67% of bivalent) as evident from almost regular meiosis shown by the hybrid plants. The homology is thus highest between these taxa (Table 2). As the pollen fertility (Table 2) is an indication of the extent of close relatedness of each pair of taxa with in the genus, it is evident that there is a strong phylogenetic affinity among *G. abyssinica, G. scabra* ssp *schimperi, G. scabra* ssp *scabra G. villosa,* and Chelelu population (Kifle Dagne, 1994, 2001a).

Hybrid plants could be produced between several taxa of *Guizotia.* However, the viability and even the formation of seed set vary from species to species combinations even when maternal and paternal crosses are reciprocated. For instance, in the cross between *G. scabra* ssp *schimperi* (maternal) and Chelelu (paternal), two hybrid plants were obtained. However the reciprocal cross-failed to produce hybrid plants. In the same way, two hybrid plants were obtained from the cross between Chelelu (maternal) and *G. zavattarii* but failed to produce seed in the reciprocal cross (Kifle Dagne, 1994, 2001a). The seed set may be affected by both prezygotic and postzygotic interactions (and pollen viability of the hybrid) (Tiffin *et al.*,

2000). Furthermore, the morphology of the hybrid plants is found to be intermediate between the parents (Kifle Dagne, 1994, 2001a).

From meiotic behaviour and chromosomal studies, it was suggested that *G. abyssinica* is probably derived from *G. scabra* ssp *schimperi.* On the other hand, *G. scabra* ssp *schimperi* strongly hybridised with the new population called Chelelu (as sexual hybrid can easily be made between cultivated *G. abyssinica* and its most related wild taxa of the genus) (Kifle Dagne, 1994, 1995, 2001a; Getinet and Sharma, 1996). Such different genomic affinity between different taxa of the genus help in the crop improvement programmes through introgressive hybridisation (and transgenic crop technology) (Ellstrand *et al.,* 1999; Jenczewski *et al.*, 2003)

Table 2. Possible crosses, chromosome pairing, pollen fertility and seed set in the hybrid plants of *Guizotia*

No.	Crosses	15 Bivalent (%)	Pollen fertility (%)	Seed set (%)
1	*G. abyssinica* vs *G. scabra* ssp *schimperi*	95	81.47	41
2	*G. scabra* ssp *schimperi* vs Chelelu	99.67	67.7	>90
3	*G. scabra* ssp *schimperi* vs *G. scabra* ssp *scabra*	59.6	49.30	61.9
4	Chelelu vs *G. scabra* ssp *scabra*	91.67	19.41	>90
5	*G. scabra* ssp *scabra* vs *G. villosa*	83	47.39	82.46
6	*G. abyssinica* vs *G. scabra* ssp *scabra*	69	46.59	19.46
7	*G. scabra* ssp *schimperi* vs *G. villosa*	57.4	34.94	46.15
8	*G. zavattaii* vs *G. arborescens*	96.	31.39	*
9	*G. abyssinica* vs *G. villosa*	88.6	30.57	19.9
10	*G. zavattari* vs Chelelu	52.4	2.35	*

* Recoded document are not available

Source: Kifle Dagne, 1994, 2001a

Guizotia abyssinica shows self incompatible out crossing behaviour and insects, particularly bees, are the major agents of pollination (Sujatha, 1997; Getinet and Sharma, 1996; Kifle Dagne, 1994, 2001a; Kandel and Lake, 2002). Some chance of *G. abyssinica* that could be self-compatible within the gene pool cannot be denied (Riley and Belayneh, 1989; Getinet and Sharma, 1996). So far, there were no report that confirm whether the wild taxa of *Guizotia* are self-compatible or not. However, there is an indication of self-incompatibility similar to that of *G. abyssinica* (Kifle Dagne, personal communication).

2.3.7. Evolutionary trends and phylogenetics in the genus

According to Baagoe (1974) the evolutionary trends and phylogenetic relationships in *Guizotia* indicate that *G. scabra* ssp *scabra* has primitive morphological features such as sessile leaves, perennial habit, and numerous florets whereas *G. reptans* and *G. arborescens* contain advanced morphological characters (e.g., petiolated leaves, and few floret). While the other species are found intermediate between primitive and advanced morphological characters. She also suggested that *G. villosa* was probably derived from *G. scabra.*

The extents of gene flow between different taxa help to show the evolutionary relationship in the genus. Most closely related group of taxa show considerable exchange of genetic material through interspecific hybridization (Murthy *et al.,* 1993; Kifle Dagne, 1994, 2001a; Ellstrand *et al.,* 1999; Jenczewski *et al.*, 2003). *G. abyssinica, G. scabra* ssp *scabra, G. scabra* ssp *schimperi, G. villosa* and Chelelu are group of taxa that share a large degree of homology and hence cannot be separated by cross incompatibility barrier (Kifle Dagne, 1994; 1995; 2001a). Esayas (1999) point out that the RFLP with the three restriction enzymes (BamHI, ECORI, and Hind II) show no variation among *G. abyssinica, G. scabra* ssp *scabra* and *G. scabra* ssp *schimperi*. On the other hand, the closer relationship of *G. scabra* ssp *scabra* to *G. villosa* (Table 2) than *G. scabra* ssp *schimperi,* as revealed by hybridization, contradicted to the basis of taxonomic proximity between the two species (Kifle Dagne, 1994, 2001a).

The karyotypic study data of *Guizotia* is also in line with the hybridization properties of the taxa. Species /populations with similar karyotypes are closely related in their phylogenetic affinity (Kifle Dagne, 1994, 2001a). The differences in the karyotypes of related species are

the result of karyotypic evolution that occurred through time (Holmoquist and Daneis 1989: cited in Kifle Dagne, 1994). Predominant evolutionary trend in Karyotype of seed plants is change from symmetry to increasing asymmetry. Increasing karyotype asymmetry is brought about by unequal translocations and pericenteric inversions. Thus symmetrical karyotypes are considered to be primitive and often associated with primitive morphological characters and asymmetrical karyotypes as derived ones. Asymmetrical Karyotype in taxa like *G. arborescens, G. reptans* are associated with advanced characters such as petiolated leaf than symmetrical karyotype (e.g.Chelelu, *G. abyssinica*) (Hiremath and Muthy, 1992). Thus, Karyotype is more reliable indicator of the direction of evolution in combination of other data such as morphological, crossability and isozyme divergence.

The cytological and hybridization studies strongly support that *G. abyssinica* was probably derived from *G. scabra* ssp *schimperi* (Baagoe, 1974; Hiremath and Murthy, 1988; Kifle Dagne, 1994, 1995, 2001a). The endemicity of the suggested progenitors in Ethiopia strengthened Ethiopia as a centre of domestication of the cultivated taxon. The pollen fertility of hybrid plants of Chelelu and *G. scabra* ssp *schimperi* (Table 2) show their close relatedness where they share immediate common ancestor even than *G. scabra* ssp *scabra* (Kifle Dagne, 2001a)

According to Kifle Dagne (1995), since the karyotype of *G. villosa* and *G. scabra* ssp *schimperi* are quite dissimilar, the former more probably evolved from *G. scabra* ssp *scabra* since there is strong similarity of karyotype and chromosomal pairing in hybrid of *G. villosa* and *G. scabra* ssp *scabra* which is in line with Hiremath *et al.*(1992). The same studies confirmed that *G. arborescens* and *G. zavattarii* are closely related to each other, but distantly related to other taxa of the genus. Because these taxa showed low chromosomal pairing, pollen fertility in the hybrid plant and variation in chromosomal morphology (Table 2) with other related taxa of the genus (Kifle Dagne, 1994, 1995, 2001a)

2.3.8. Crop improvement potential in niger

Farmers in Ethiopia, plant the different landraces in different seasons depending on the regional climatic condition (Getinet and Sharma, 1996). Niger yield has a low response to nitrogen and phosphorous fertilizer that have an effect only on vegetative part. Niger seeds are loosely held in the head threshing. Thus the plant developmental stage at harvest and the variety planted are found to be important factors contributing to the seed yield (Getinet and Sharma, 1996). Four improved varieties (Sendasa, Fogera-1, Esete-1 and Kuyu) have been released in Ethiopia to increase seed yield (Getinet and Sharma, 1996). However, the breeding efforts have been limited to Ethiopia and India (Riley and Belayneh, 1989), the Ethiopian varieties attract the US agricultural industry and as a result the research efforts even have increased abroad (Kandel and Lake, 2002; Quinn and Myers, 2002).

Dwarf types of cultivars have been developed to reduced plant height, which would decrease the number of leaves that contribute for crop improvement (Getinet and Sharma, 1996). Another potential for improvement of niger is the greater genetic variability for the oil content that could be used in breeding programme to produce high quality oil (Getinet and Adefris, 1995).

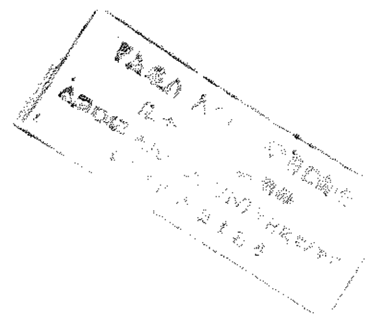
As a result of self-incompatible nature of this crop, cross-pollination through recurrent mass selection is a mandatory approach (Agrawal, 1998). The identification of male sterility opened the way for exploitation of heterosis in niger. Even if there is no as such observed heterosis on oil content yet, around 10-30% heterosis seed yield over better parent and 15-55% over the mid parent could be acquired (Getinet and Sharma, 1996). Generally, genetic variation in crops and their wild relatives hold the key to the successful breeding of improved crop cultivars with durable resistance to disease. Similarly adequate race survey would enable identification of various races, which are immediate concern in the crop improvement programmes (Agrawal, 1998; Quinn and Myers, 2002)

2.3.9. Agronomy of niger

Since niger is cultivated in both temperate and tropical climates, it shows adaptation to a semi tropical environment. It prefers moderate temperature for growth (19^0C to 30^0C) and even temperature of 9^0C has no effect on growth (Getinet and Sharma, 1996). The reported yield of niger seed varied from 250 to 500 kg/ha (Riley and Belayneh, 1989). The Ethiopian types of niger are of three maturity groups and tend to be shorter, more branched, and tolerate cooler conditions than the Indian types (Getinet and Sharma, 1996; Kandel and Lake, 2002; Quinn and Myers, 2002). In Ethiopia, more than 95% of Noog production comes from the province of Gojam, Gonder, Wellega and Shewa, where it grows at altitudes of 1500-2400 m above sea level. Noog often grow with inter cropping (with Sorghum) or in rotation with cereal teff, on poorly drained heavy clay soils (Getinet and Adefris, 1995; Mulatu Geleta *et al.*, 2002). To increase the yield in different agroecological zones of the country, the research in relation to climate factors and its effect on Noog seed yield have been conducted in different research centre (Adefris and Girma, 2002)

2.3.10. Biotechnological advances in niger

Despite the reduced fertility of autopolyploid niger plants, artificially induced autopolyploids of *G. abyssinica* produced a large size seeds that might increase the yield of the crops through successive selection (Kifle Dagne, 2001b). Modern techniques of plant tissue culture (in vitro shoot regeneration) of niger (Sujatha, 1997), double haploid through anther culture (Sarvesh *et al.,* 1994) help to produce homozygote line with desirable traits. Furthermore, development of transgenic niger plants through transformation with Agrobacterium infection provides a great potential for gene transfer from wild relatives to the Noog plant (Getinet and Sharma, 1996; Murthy *et al.*, 2003). All of these provide an input for the development of transgenic niger crops that develop resistant to disease such as leaf spot, stem and leaf blight and insect resistant such as Niger fly in the near future.



On the other hand, the current studies on induction of male sterility in crop plants using gametocides is of economic importance since it facilitates production of F_1 hybrids for the exploitation of hybrid vigour and highly productive recombinants (Gangaprasad *et al.*, 2004).

Even though niger breeding effort had been limited to Ethiopia and India (Riley and Belayneh, 1989), the internal and external demand of niger for oil industry, bird feed market that increased and consequently the limited research activities in the country extended to abroad (US) (Quinn and Myers, 2002; Kandel and Lake, 2002). Thus, niger has a greater potential to be improved through conventional and modern agricultural (or transgenic) technology in the near feature of crop improvement programme (Quinn and Myers, 2002; Kandel and Lake, 2002).

2.3.11. Oil content of Guizotia

The genus *Guizotia* contains oil that could be edible (Kifle Dagne and Johnson, 1997). The oil content of *Guizotia abyssinica* is higher (416-436 gm/kg weight per kg dry matter of seed) than that of the wild taxa (214-328 gm/kg). However, the fatty acid composition of all the taxa is very similar (Kifle Dagne and Johnson, 1997). On the other hand, the fatty acid composition of the Ethiopia and Indian niger show difference. The Ethiopian niger oil contains about 20% higher linoleic acid and 20% lower oleic acids than the Indian niger oil (Getinet and Sharma, 1996; Kandel and Lake, 2002). Kifle Dagne and Johnson (1997) found two unsaturated fatty acids [linoleic (54.3 – 72.8, weight percent of total lipid) and oleic (5.4 – 9.68%)], and two saturated fatty acids [palmitic (7.8 – 10%), and stearic (5.5 – 8.1 %)], which formed about 91- 97% of the fatty acids present. The amount of oleic acid in G. *scabra* ssp *scabra* and *G. scabra* ssp *schimperi* is higher (10.3 – 12%) than that of *G. abyssinica* 5.4 – 7.5%) (Kifle Dagne and Johnson, 1997). On the other hand the total lipid of niger seed accounts 49.9% of which 97% is neutral lipid (Ramadan and Morsel, 2002).

The feasible advantage of the similarity in fatty acid composition of the wild and cultivated form of *Guizotia* is that genes for desirable traits could be transferred from wild species to the cultivated taxa without considerable effects on its oil quality (Kifle Dagne and Johnson, 1997; Agrawal, 1998). As Getinet and Adefris (1995) pointed out, there is a significant

variability in oil content in different Noog cultivars: consequently, its content at least could be increased by 5% through simple selection. This indicates that there is a high variability existing within the crop for desirable agronomic traits to increase the quality of the oil seed as a whole.

2.3.12. Economic contribution of Guizotia

The economic contribution of *Guizotia* lies on the cultivated *G. abyssinica* (niger). Niger seed is the most important oil crop in Ethiopia and minor crop in India, but is not involved in the worldwide oil seed trade (Quinn and Myers, 2002). Niger seed provides 50–60% of Ethiopian's indigenous edible oil but only 2% of India's total oil seed production. It represents also minor oilseed crop in some other African countries (Riley and Belayneh, 1989). Besides cookery, niger seed oil can be used in the manufacture of soap, paints, lubricant or illuminate (Kifle Dagne and Johanson, 1997). The protein rich meal (33%) (Residue after extraction) is used for feed, manure or fuel. Niger seed also provide a food for birds (Quinn and Myers, 2002; Kandel and Lake, 2002), traditional medicine (Mulatu Geleta *et al.*, 2002), and a media for growth of fungi. Although, niger seed has low productivity that could be associated with its inherent self-incompatibility or genotype and environmental interaction on yield determining traits, the current level of the niger seed market coupled with the high cost of niger seed ($ 1.10/kg for Niger seed compared to 0.26/kg for sunflower) indicates that the market potential is great (Quinn and Myers, 2002). However, at lower prices and yields niger seed may not provide a reasonable agronomic alternative to traditional crops.

2.4. Use of genetic marker in the study

There are a number molecular techniques currently used in population genetic study including isozyme, RAPD, AFLP among others. Which technique is most appropriate for a particular question depends up on (1) the extent of genetic polymorphism required to best answer the question, (2) the analytical or statistical approach available for the technique's application and, (3) the practical knowledge of time and costs of materials (Murthy *et al.*, 1996; Parker *et al.*, 1998)

The systematic and phylogenetic relationship among plant taxa traditionally is dependent on the comparative analysis of phenotypic characters that are subjected to phenotypic plasticity or the dynamic environment (Murthy *et al.*, 1996). Molecular level of analysis is increasingly provided detailed and often unexpected evidence of specific relationship among different taxa (Wendel and Weeden, 1989; Murthy *et al*, 1996; Parker *et al*, 1998).

2.4.1. Isozyme as a genetic marker

The migration of proteins under the influence of an electric field is among the most cost effective methods of investigating genetic phenomenon at the molecular level (Murthy *et al.*, 1996). Living organism often produces multiple forms of enzymes, that are different molecular forms catalysing the same chemical reaction may be found in the same individual (Wiley and Sons, 1990). Such molecular forms of an enzyme encoded by different alleles of the same gene locus are termed as allozymes. The different molecular forms of enzymes encoded by different loci more than one gene are referred as isozymes (Wiley and Sons, 1990; Parker *et al.*, 1998; Wendel and Weeden, 1989).

Allozymes are more convenient and reliable genetic markers that exhibit Mendelian inheritance, codominant expression, complete penetrance and absence of pleiotrophic and epistatic interactions (Wendel and Weeden, 1989; Murthy *et al.*, 1996). Most interesting of all properties of isozymes is the simple genetic basis of most polymorphism. Isozymes are proteins that can directly reflect alteration in the DNA sequence that changes the amino acid sequences in a given protein (Wiley and Sons, 1990; Murthy *et al.*, 1996; Parker *et al.*, 1998). Such changes produce a change in electrophretic mobility (Wiley and Sons, 1990; Murthy *et al.*, 1996). Even if the possibility of amino acid substitutions is not affecting the over all function of the protein, change in the elecrophoretic mobility of enzymes provide an extremely useful method of evaluating genetic differences among groups (Wendel and Weeden, 1989). The more recent findings of introns within structural genes and existence of multigene families for many proteins have not seriously undermined the ability of isozyme polymorphism to serve as a direct measure of DNA sequence within and among genomes (Murthy, *et al.*, 1996)

2.4.2. *Selective advantage of isozyme marker*

Besides its cost effectiveness, the observation of condominant expression in nearly all cases examined provides compelling evidence that both alleles are transcribed and both transcripts are translated so that the marker provides a direct method of visualizing the products of both alleles (Wendel and Weeden, 1989; Wiley and Son, 1990; Kephart, 1990; Murthy *et al.*, 1996). Furthermore, comparable data from previous studies and a wealth of standard statistical procedures make isozyme markers appealing for studies of both fine and broad scale genetic variation (Parker *et al.*, 1998)

Generally, using enzymatic and non-enzymatic proteins, numerous investigations have focused on enzyme efficiency, estimating and understanding genetic variability in the natural population, gene flow, and phylogenetic relationships among others (Wendel and Weeden 1989; Murthy *et al.*, 1996; Parker *et al.*, 1998).

3. Objectives

General objective: To investigate the evolutionary relationship between *Guizotia abyssinica* and other species or subspecies/taxa of the wild forms

Specific objectives:

- To estimate the genetic variability within and among each taxa using isozyme data.
- To decide which of the wild relative of *Guizotia* is the possible progenitor of *Guizotia abyssinica* based on isozyme data

4. Material and Methods

4.1. Plant material

Seeds of twelve populations of *Guizotia*, representing each taxon were sampled from large collections previously made from different regions of agroecological zones by Mulatu Geleta (a Ph.D student at A.A.U) plus Chelelu population that was collected by ourselves were used (Table 3). The representative populations for selected regions were sampled in two ways. (1) One population randomly pick up from each selected regions so that four populations of the cultivated type became ready for examination. (2) As some of wild species /population had small number of accessions /populations, we selected in such a way that represent the different regions of the country as much possible as we could to make low probability of gene flow to the adjacent regions for both cultivated and wild types. Each of the collected population comprised 10 to15 groups of seeds each having the same maternal origin. Seeds from each maternal origin were germinated on filter paper in separated petridishes at room temperature after 8 hours of incubation at 37^0C to increase rate of germination. After the seedling reached two-leaf stage, one individual seedling randomly selected from 5-10 seedlings having same maternal origin used for examination. A total of 9 to 11 individual seedlings with different maternal origin per population were ground in a general extraction buffer for enzyme assay (Kephart, 1990; Murthy *et al.*, 1996; Chamberlain, 1998).

4.2. Enzyme systems and tissue type

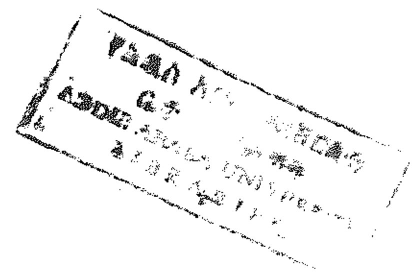
Three enzyme systems were established based on the availability of the required chemicals and their polymorhic nature. aspartate aminotraniferase (AAT, E.C. [Enzyme Commission number] 6.1.1.), acid phosphatase (ACP, C.E. 3.1.3.2.) and phosphoglucoisomerase (PGI, E.C. 5.3.1.9.) after extensive testing of these enzyme system with different seedling stages, part of a seedling, extraction buffer, tissue type; buffer ratio, Gel buffer, PH, wettage, duration of electrophoresis and staining procedures to get better resolution based on Chamberlain (1998) and Wendel and Weeden (1989) protocols. Relatively better resolution from four days seedling of *Guizotia abyssinica* and 8-12 days for the wild type of the genus was used. The wild *Guizotia* showed relatively lower rate of germination even after heat

enzyme assay. A total of 131 individual plants were examined in this project (Table 3).

Table 3. Location (origin) of the 13 *Guizotia* populations and the sample size used for isozyme analysis.

No.	Population	Call. Code	Sample size	Origin (Region/Wereda)	Altitude	Remark
1	*G. abyssinica*	C_{25}	10	Arsi/shirka	2380	Cult. *
2	*G. abyssinica*	B_{15}	9	Gonder/Adi Arkay	1890	Cult
3	*G. abyssinica*	G_2	9	Illubabor/Gachi	2045	Cult
4	*G. abyssinica*	F_4	10	Shewa/Woliso	2372	Cult
5	*G. scabra* ssp *schimperi*	J_7	10	Shewa/Ifat&Gidim	1600	Wild
6	*G. scabra* ssp *schimperi*	W_7	10	Welo/Dassie Zuria	2320	Wild
7	*G. villosa*	A_{20}	10	Tigray	2410	Wild
8	*G. villosa*	B_{16}	10	Gojam	1920	Wild
9	*G. scabra* ssp *scabra*	B_{11}	10	Tigray	2300	Wild
10	*G. scabra* ssp *scabra*	E_1	10	Sidamo	1890	Wild
11	*Chelelu*	U_7	11	Shewa/Sendafa	2475	Wild
12	*G. arborescens*	B_{200}	11	Jimma (Omonada)	2375	Wild
13	*G. arborescens*	I_{240}	11	Jimma (Chida)	2200	Wild

* Cultivated species



4.3. Gel and electrode buffer preparation

Although many different buffers exist, we had found that only a few different ones were required to sufficiently resolve a large number of enzymes for widely divergent organisms (Kephart, 1990). Extensive trials were conducted with different buffer systems at different PH to resolve all selected enzymes equally. Lithium borate buffer system at a PH of 7.6 became a buffer of choice. The gel buffer, which comprised 0.042M trisbase, 0.007M citric acid, monohydrate, 0.004M lithium hydroxide, 0.025M boric acid per litre of deionized water at PH of 7.6. Electrode buffer contains 0.039 Lithium hydroxideand 0.263 boric acid. In both buffers the PH was adjusted with 5M NaOH and hydrochloric acid (Kephart, 1990; Chamberlain, 1998). Enough quantity of this buffer system was prepared and stored at $4^{o}C$ to be used through out the experiment.

4.4. Starch gel preparation

Starch-gel was prepared in the afternoon using 11% hydrolysed potato starch (made of USB Corporation in USA). We boiled approximately three-fourth of the total gel buffer volume desired in 500 ml flask in microwave oven and added onto the remaining cold buffer with its suspended starch (Kephart, 1990; Weeden and Wendel, 1989; Chamberlain, 1998). The mixture was heated and swirled vigorously until forming a colloid, followed by degassing using degassing electric machine (Vacuum pump). Immediatèly pouring the degassed gel into a levelled gel mould. After 30-40 minutes the gel was covered with plastic wrap and put on over the night in the cabinet at room temperature to be used on the next day. On the next day the gel was kept in the refrigerators 40-60 minutes to keep the sample inserted in the gel as cold as possible during electrophoresis. (Weeden and Wendel, 1989; Kephart, 1990; Chamberlain, 1998).

4.5. Sample preparation

Shoots of two-leaf stage seedling from each population were ground in 1-2 drops per sample with general extraction buffer. The general extraction buffer comprised 50 ml lithium borate gel buffer (PH=7.6), 37 mg potassium chloride, 10 mg magnesium chloride, 18 mg EDTA (disodium), 25 mg pvp-40, 0.5 ml Triton-X-100 and 2 ml 2-mercaptoethanol per 50 ml of extraction buffer. Extraction of tissues carried out on Plexiglass sample plate with tip round glass rod fit on wells of plate. The tissues extraction protocol was adapted from Chamberlain (1998). Care had been taken to avoid cross contamination from neighbour sample wells during extraction. All steps were conducted under cold condition (on ice) to preserve enzyme activities.

4.6. Sample loading

Extracts were absorbed onto 3 mm wicks of whatman filter paper and immediately loaded on gel after blotting both surface of wick on filter paper to prevent diffusion between adjacent wicks. Bromophenol blue marker absorbed with wicks also loaded at the right and left end 3 mm inside from the edge of the gel and between populations to control the electrophoretic mobility during electrophoresis (Kephart, 1990; Chamberlain, 1998).

4.7. Electrophoresis

We placed the gel mould into the electrophoretic apparatus and filled each half of the apparatus with running buffer. The gel and running buffer connected with spongy clothes by overlapping with 3 cm onto the gel and covered with plastic wrap. We placed ice onto acrylic tray on the top of apparatus to keep cool during electrophoresis. Electrophoretic power supply was used to run the gel with constant amperage (70 mA, 250 V). The electrophoresis was continuously monitored until the bromophenol blue tracker dye had migrated 8cm from the origin (about 6-8 hrs) (Kephart, 1990; Chamberlain, 1998).

4.7.1. Stain preparation

The stain recipes were prepared in advance according to Wendel and Weeden (1989) and Chamberlain (1998) that comprised 100 ml 0.4 M sodium acetate buffer, PH 5.0 (pre-soak buffer) and 100 ml 0.2 M sodium acetate buffer PH 5.0 (stain buffer), 100 mg B-napthyl acide phosphate, 100 mg Fast Garnet GBC salt and 1 ml 10 % magnesium chloride for acid phosphatase; 100 ml 0.1 mTris-HCl PH 8.5, 36 mg α-Ketoglutaric acid, 130 mg L-aspartic acid, 500 mg pvp-40T, 100 mg disodium EDTA, 1.42 mg Na_2HPO_4 and 400 mg fast blue BB salt for aspartate aminotransferase ;100 mg 0.1M Tris-HCl P_H 7.5, 40 mg disodium fructose-6-phosphate, 40 unit Glucose-6-phosphate dehydrogenate,14mg NADP, 24mg MTT, 6 mg PMS and 1 ml 10 % $MgCl_2$ for phosphoglucose isomerase .

4.7.2. Gel slicing and staining

When tracking dye reached 8 cm away from the origin (after 6-8 hrs), we stopped the gel run and placed the gel on a dry acrylic slicing bed. Eight slicing Guides with 1.5mm thick and thin tightened wire were used for slicing the gel. Then after, each of the sliced gel put on the staying tray and poured the recipes for respective enzyme system AAT, PGI and ACP based on Wendel and Weeden (1989) and Chamberlain (1998) protocols. After staining completed the slice immediately fixed with 50% glycerol for all enzyme systems.

4.7.3. Scoring and photographing gel

The zymograms of AAT and ACP enzyme systems kept in the refrigerators over night at 4^0C while PGI scored and photographed after three hours of fixation. On the next day morning we scored the remaining zymogrames and rechecked the scored ones by wrapping the gel with transparent thin plastic followed by photographing with different lens apertures using Biometra Analysers (Bio Doc Analyze) for further recording and interpretation (Wendel and Weeden, 1989; Kephart, 1990; Chamberlain, 1998). The locus encoding the most anodal migrating genes product was designated as 1, the next 2, etc. Similarly, the most anodal migrating allozymes of a given gene was assigned with ‘A’ whereas subsequent letters indicated slower migrating proteins.

4.8. Data analysis

As *Guizotia* is diploid and open pollinated, genetic interpretation of isozyme gel banding patterns were based on the evaluation of isozyme polymorphisms in other well documented investigations of related plant species (Wendel and Weeden, 1989; Kephart, 1990). Knowledge of the number of loci involved, enzyme subunit structure, and subcellular localization in other species provided an input for the interpretation of stained zymograms (Wendel and Weeden, 1989)

The methods of Nei (1978) were employed to compute gene diversity statistic. Proportion of polymorphic loci, mean number of alleles per locus and observed and expected proportion of heterozygous loci were calculated for the entire populations, pooled and grouped populations at species/taxa level of *Guizotia* using Popegene Program. Chi-square test was employed to determine whether observed value differ from those expected under Hardy Weinberg equilibrium; 5% significance level was used.

The gene diversities per locus and over all loci for each population and species/taxa were calculated. The deviation of observed heterozygote frequencies from Hardy–Weinberg expectations for each polymorphic locus in each population and species/taxa were estimated using Wright's (1978) fixation index. Total genetic diversity was partitioned (grouped) and calculated within and among populations of species/taxa to assess genetic differentiation at species/taxa level following Nei (1987).

The following estimating parameters were used in our data analysis.
Expected hetrozygosity (H_{exp}) for a single locus is =

$$1-\sum_{i=1}^{k} p_i^2$$

Where p_i is the frequency of the ith of k alleles

The average gene diversity (H_{exp}) over several loci is =

$$1-\frac{1}{m}\sum_{l=1}^{m}\sum_{i=1}^{k}p_i^2$$

Where the first summation is for the lth ("alleleth") of m loci

F_{IS} (inbreeding coefficient) = H_{exp} - H_O / H_{exp} where H_O is the observed hetrozygosity and H_{exp} is the expected hetrozygosity estimated on the assumption of random mating.

F_{ST} (genetic differentiation) = $(H_T - H_S)/H_T$, where H_T is the expected hetrozygosity for a pooled (grouped) sample of alleles at species level and H_S is the average expected hetrozygosity $(1-\sum(Pi)^2$ within each population

F_{IT} (the overall inbreeding coefficient) = $(H_T - H_O) / H_T$.

Gene flow (Nm) = $0.25(1-F_{ST})/F_{ST}$

Total gene diversity:

Between population (D_{ST}) = H_T-H_S

Among populations (G_{ST}) = D_{ST}/H_T

The genetic distance between population (D) = **-ln (I)** where I = Jxy/√ (Jxx) (Jyy), Jxy = P (two alleles chosen from different populations [x and y] are the same); Jxx, Jyy = P (two alleles chosen from the same population [x and y] are the same).

Two-level hierarchical analysis was carried out on allele frequencies. The variance components were computed: within and among population and pooled (or grouped) populations at species/taxa level. A UPGMA (Un weighted Pair Group Method with Arithmetic averaging) phenogram was produced by input of Nei's (1978) genetic distance values into the POPEGENE VERSION 1.31 MICRO SOFTWARE program written by Yah and Yang, (1999) at University of Alberta.

5. Result

5.1. Relative electrophoretic mobility and allozyme pattern

5.1.1. Relative electrophoretic mobility

Eight isozyme loci were found in three enzyme systems. They were AAT-1, AAT-2, AAT-3, ACP-1, ACP-2, and PGI-1, PGI-2, PGI-3. The eight scored loci gave a total number of 48 alleles, which are summarized in Table 4. One locus (PGI-3) was restricted to only one taxon (specific) and the rest loci were found in all taxa surveyed. All the material surveyed has three AAT-regions with different number of bands and various rate of electrophoretic mobility (Figure 1). They were named as AAT-1, AAT-2 and AAT-3 from fastest to slowest migration rate toward the anodal regions of the zymograms. The AAT-1 band region of population I_{240} and B_{200} displayed the fastest mobility and hence most near to the anodal regions. Considerable migration differences were also observed between the cultivated and wild type of *Guizotia.*

Most bands of AAT-1 in *G. abyssinica* were relatively near to anodal regions next to *G. arborescens* (I_{240} & B_{200}). The AAT-2 bands of the wild populations B_{11}, $E_{1,}$ A_{20} and B_{16} had higher rate of mobility than the wild populations J_7 and $W_{7.}$ At this locus the cultivated populations (C_{25}, $G_{2,}$ F_4 and B_{15}) and the wild type (U_7) showed slow electrophoretic mobility where as populations I_{240} and B_{200} displayed the fastest mobility in the zymograms. At AAT-3 locus, most of the wild and cultivated populations showed medium migration rate and they were almost on one level of starch gel except two populations of *G. arborescens*. In this locus *G. arborescens* displayed slower migration rate relative to the other surveyed genotypes.

The bands in ACP-1, ACP-2, PGI-1 and PGI-2 were found at the same level with some interspecific and intraspecifc electrophoretic mobility differences on starch gel in all studied materials. The PGI-3 was unique for *G. arborescens* (I_{240} & B_{200}) and produce hetrodimer band with PGI-2 locus (Figure 3).

5.1.2. Allozyme pattern

AAT

Three zones of activity considered as products of three loci (AAT-1, 2,3) were observed in all populations in line with the most closely related species (Cronn *et al.,* 1997; Crawford *et al.,* 1984). In AAT-1 two populations (I_{240}, B_{200}) were fixed with allele A. Allele B and alleles C were present in all other populations/species. Population U_7 had a number of missing data probably due to gene silencing or faint staining that cause miss-scoring. At this locus, the domesticated *Guizotia* was strongly stained as in Mulatu Geleta (2001). At AAT-2 allele D was found in population G_2, F_4, U_7, J_7 and fixed in populations C_{25} and B_{15}. Allele E displayed in G_2, U_7 and with the least frequency (0.05) in population F_4. In this locus allozyme B was found in population B_{16} and fixed at both populations A_{20} and E_1. The third type of allozyme (allele C) was found only in populations B_{16} and J_7. In the third locus (AAT-3) population G_2, F_4, B_{15}, U_7 and W_7 were fixed with allele B and found in most surveyed populations from lowest frequency (0.333) to highest one (fixed) (Table 4 and 8). Allozyme C was found in C_{25}, U_7, J_7, E_1 and B_{16} from lowest frequency (B_{16} = 0.05) to highest frequency (E_1 = 0.667).

ACP

It was interpreted as a monomeric enzyme with two loci showing two bands for heterozygote and one band for homozygote. The most anodal band (ACP-1), although weakly stained could be scored. At this locus (ACP-1) population B_{11} and A_{20} was fixed with allele A. Allozyme A and B were present in all surveyed populations. In both ACP-1 and ACP-2 loci allele C was found in lower frequency (0.056- 0.150) in B_{15}, C_{25}, B_{15} and W_7 that could be considered as rare allele in the populations surveyed (Figure-2, Table 4).

PGI

Four zones of activity were observed in populations I_{240} and B_{200} and two zones of activity displayed in the rest of the populations normally found in diploid plant (Bayer *et al.*, 1986; Crawford *et al.,* 1984; Cronn *et al.,* 1997). PGI is a dimeric enzyme each locus showing three alleles. The most anodal bands (PGI-1) though weakly stained could be scored easily. The two types of allozyme (A&B) present through out the population under study. In the locus PGI-2 allozyme A and B were common and distributed uniformly. Two populations (I_{240} & B_{200}) were fixed with allele A. Allozyme C was found in populations B_{15}, U_7, W_7, J_7,

Unlike all populations surveyed PGI-3 was found only in populations I_{240} and B_{200} and fixed with A allele. The third band between PGI-2 and PGI-3 displayed could be the product of these two loci that form hetrodimer. This locus and the hetrodimer make these populations unique from all other studied materials (Figur-3). In the following figures, rows indicated for direction of mobility from cathodal to anodal region, numbers indicated above the Zymograms show lanes for each population and L1-3 denoted for loci.

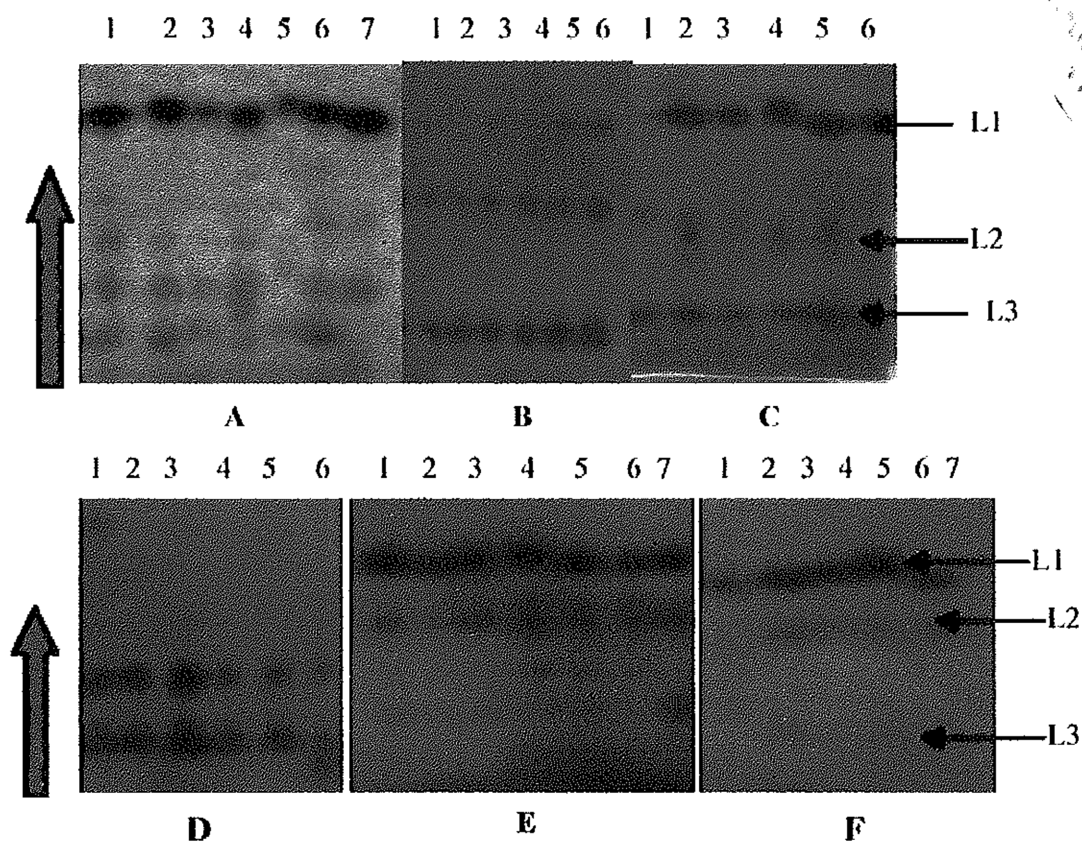


Figure 1. Zymograms of AAT on starch-gel. A: *G. abyssinica* (C25), B: *G. arborescens* (I240), C: *G. scabra* ssp *schimperi* (J7), D: Chelelu (1-6), E: *G. villosa* (1-4), F: *G. scabra* ssp *scabra* (E).

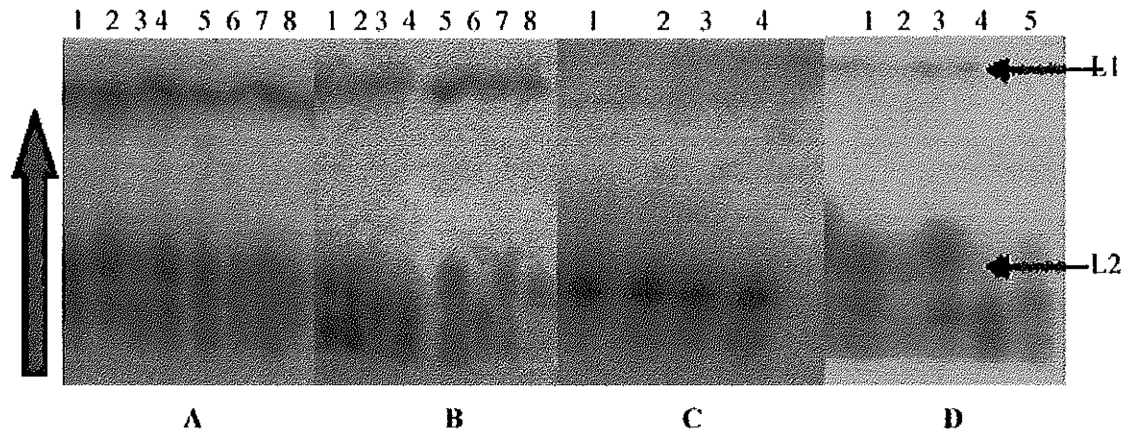


Figure 2. Zymograms of ACP on starch-gel. A: Chelelu (1-4), *G. scabra* ssp *schimperi* (5-8), B: *G. villosa* (1-4), *G. scabra* ssp *scabra* (5-8), C: *G. arborescens* (1-4), D: *G. abyssinica* (1-5)

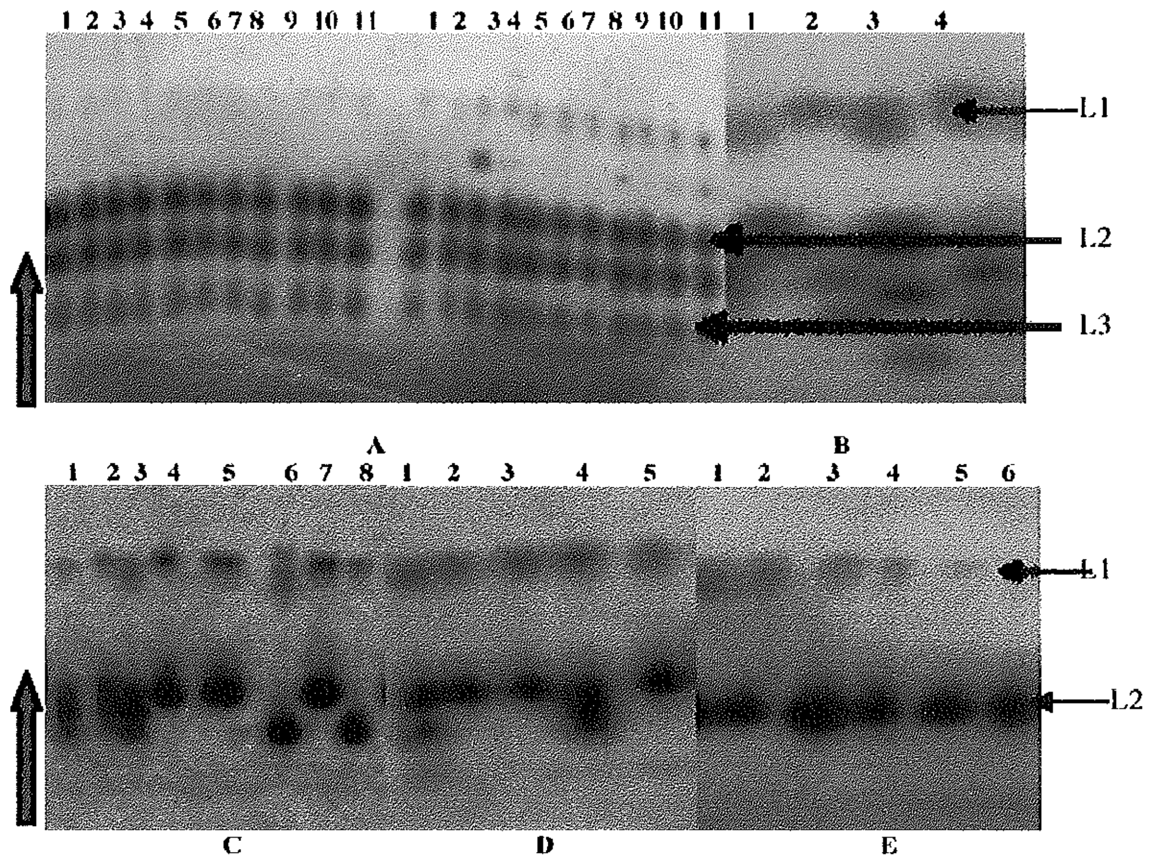


Figure 3. Zymograms of PGI on starch-gel. A: *G. arborescens, B: G. scabra* ssp *schimperi* (1-4), **C:** *G. villosa* (1-4) and *G. scabra* ssp *scabra* (5-8), **D:** *G. abyssinica* (1-5), E**:** Chelelu (1-6)

Table 4. Allelic frequencies of 13 populations of *Guizotia* species. Pop. denoted for population, AL for alleles per locus

Pop	AL	AAT-1	AAT-2	AAT-3	ACP-1	ACP-2	PGI-1	PGI-2	PGI-3
C_{25}	A	0.0000	0.0000	0.0000	0.5360	0.6500	0.8000	0.7500	0.0000
	B	0.6500	0.0000	0.9000	0.4440	0.2500	0.2000	0.2500	0.0000
	C	0.3500	0.0000	0.1000	0.0000	0.1000	0.0000	0.0000	0.0000
	D	0.0000	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
G_2	A	0.0000	0.0000	0.0000	0.1429	0.5000	0.7222	07778	0.0000
	B	0.7222	0.0000	1.0000	0.8571	0.5000	0.2778	0.2222	0.0000
	C	0.2778	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	D	0.0000	0.8750	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	E	0.0000	0.1250	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
F_4	A	0.0000	0.0000	0.0000	0.6875	0.3500	0.9000	0.6000	0.0000
	B	0.6000	0.0000	1.0000	0.3125	0.6500	0.1000	0.4000	0.0000
	C	0.4000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	D	0.0000	0.9500	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	E	0.0000	0.0500	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
B_{15}	A	0.0000	0.0000	0.0000	0.5625	0.3333	.7777	0.5000	0.0000
	B	0.5556	0.0000	1.0000	0.4375	0.6111	0.2222	0.3888	0.0000
	C	0.4440	0.0000	0.0000	0.0000	0.0556	0.0000	0.1111	0.0000
	D	0.0000	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
U_7	A	0.0000	0.0000	0.0000	0.2727	0.5000	0.6000	0.1818	0.0000
	B	0.3333	0.0000	0.9000	0.7273	0.5000	0.4000	0.5455	0.0000
	C	0.6667	0.0000	0.1000	0.0000	0.0000	0.0000	0.2727	0.0000
	D	0.0000	0.8636	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	E	0.0000	0.1364	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
W_7	A	0.0000	0.0000	0.0000	0.5000	0.2000	0.6500	0.5000	0.0000
	B	0.1666	0.0000	1.0000	0.5000	0.6500	0.3500	0.2500	0.0000
	C	0.8333	1.0000	0.0000	0.0000	0.1500	0.0000	0.2500	0.0000
J_7	A	0.0000	0.0000	0.0000	0.4000	0.4500	0.6500	0.2000	0.0000
	B	0.1111	0.0000	0.9444	0.6000	0.5500	0.3500	0.3000	0.0000
	C	0.8889	0.8333	0.0556	0.0000	0.0000	0.0000	0.5000	0.0000
	D	0.0000	0.1667	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

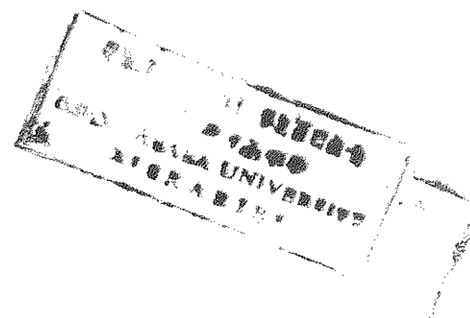


Table 4. continued

Pop	AL	AAT-1	AAT-2	AAT-3	ACP-1	ACP-2	PGI-1	PGI-2	PGI-3
B_{11}	A	0.0000	1.0000	0.0000	1.0000	0.5500	0.8000	0.5000	0.0000
	B	0.1500	0.0000	1.0000	0.0000	0.4500	0.2000	0.4000	0.0000
	C	0.8500	0.0000	0.0000	0.0000	0.0000	0.0000	0.1000	0.0000
E_I	A	0.0000	0.0000	0.0000	0.7143	0.6111	0.9500	0.4500	0.0000
	B	0.1500	1.0000	0.3333	0.2857	0.3889	0.0500	0.5000	0.0000
	C	0.8500	0.0000	0.6667	0.0000	0.0000	0.0000	0.0500	0.0000
A_{20}	A	0.0000	0.0000	0.1250	1.0000	0.0000	0.6500	0.1000	0.0000
	B	0.3000	1.0000	0.8750	0.0000	0.6500	0.3500	0.6500	0.0000
	C	0.7000	0.0000	0.0000	0.0000	0.3500	0.0000	0.2500	0.0000
B_{16}	A	0.0000	0.0000	0.1000	0.4286	0.0000	0.5500	0.0000	0.0000
	B	0.3000	0.8500	0.8500	0.5714	0.6500	0.4500	0.7000	0.0000
	C	0.7000	0.1500	0.0500	0.0000	0.3500	0.0000	0.3000	0.0000
I_{240}	A	1.0000	1.0000	0.0000	0.8636	0.5909	0.6818	1.0000	1.0000
	B	0.0000	0.0000	0.0000	0.1364	0.4091	0.3182	0.0000	0.0000
	D	0.0000	0.0000	1.0000	1.0000	0.0000	0.0000	0.0000	0.0000
B_{200}	A	1.0000	1.0000	0.0000	0.6110	0.5909	0.6364	1.0000	1.0000
	B	0.0000	0.0000	0.0000	0.3888	0.4091	0.3636	0.0000	0.0000
	D	0.0000	0.0000	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	48	24	18	19	25	29	25	31	2

5.2. Genetic variation

5.2.1. Genetic diversity within populations

The various estimated values of genetic variation both at species and population level were show in Table 5. The genotypic frequencies were tested for fitting Hardy-Weinberg equilibrium for each locus in all populations. The test showed that almost all (88%) populations and loci did not fit the Hardy-Weinberg equilibrium. Those populations and loci that were in Hardy-Weinberg equilibrium in terms of genotypic frequencies were: AAT-1 in population F_4 and J_7, AAT-2 in populations G_2 and F_4, AAT-3 in population J_7, ACP-1 in population I_{240}, ACP-2 in population B_{16}, and PGI-1 in C_{25}, F_4, E_I & U_7.

Table 5. The average number of alleles per locus (*A*), the percentage of polymorphic loci (*P*), average observed heterozygosity (H_o) and expected heterozygosity (H_{exp}) at eight loci for examined populations of *Guizotia* species

Species/taxa	Population	A	P	H_o	H_{exp}*
G. *abyssinica*	C_{25}	2.00	75.0	0.3980	0.3510
	G_2	1.86	75.0	0.3650	0.3209
	F_4	1.86	75.0	0.3460	0.3196
	B_{15}	2.00	62.5	0.4430	0.3682
Mean		***1.93***	***71.8***	***0.3880***	***0.3399***
Chelelu	U_7	**2.00**	***87.5***	***0.2860***	***0.4339***
G. scabra ssp *schimperi*	W_7	2.00	62.5	0.3160	0.3570
	J_7	2.14	87.5	0.3780	0.3960
Mean		***2.07***	***75.0***	***0.3470***	***0.3765***
G. scabra ssp *scabra*	B_{11}	1.71	50.0	0.1710	0.2481
	E_1	2.00	75.0	0.1840	0.3385
Mean		***1.86***	***62.5***	***0.1775***	***0.2933***
G. villosa	A_{20}	1.86	62.5	0.3140	0.3093
	B_{16}	2.14	87.5	0.2570	0.4227
Mean		***2.00***	***75.0***	***0.2855***	***0.3660***
G.arborescens	I_{240}	1.37	37.5	0.2160	0.1510
	B_{200}	1.37	37.5	0.2900	0.1868
Mean		***1.37***	***37.5***	***0.2530***	***0.1689***
Total mean		**1.87**	**77.1**	**0.3036**	**0.3233**

$*H_{exp} = 1-\Sigma Pi^2$

The percentages of polymorphic loci ($p<0.05$) were detected in the range from 37.5 to 87.5 with a mean of 77 in all populations assessed (Table 5). The average number of allele per locus ranged from 1.370 to 2.1429 with a mean of 1.871. Unbiased genetic heterozygosity computed following Nei (1978), the range was from 0.151 (in I_{240}) to 0.4339 (in U_7) (Table 5). The average observed and expected genetic diversity for all the populations surveyed was 0.3036 and 0.3233 respectively and these results are higher than the results of related species reported (Cronn *et al*, 1997).

The Wright fixation index (F_{IS}) is given in Table 6. The results indicated that 47.25 % of the surveyed populations in common loci (excluding PGI-3) had excess heterozygosity and nearly 29 % showed deficiency of hetrozygocity indicating that most studied population experienced with non-random mating or selection, and/or mutation or combination of these

factors. Where as the rest of the surveyed populations exhibited balanced heterozygosity.

Among domesticated populations, B_{15} (from Gonder) had greater gene diversity compared to the other populations (H_{exp} = 0.3682) within the range of 0.3196 (F_4 from Shewa/Weliso) to 0.3682 ($B_{15,}$ Gonder). The wild populations also showed gene diversity in the range of 0.151 (I_{240}) to 0.4339 (U_7). Within the wild populations I_{240} and B_{200} showed the least genetic diversity where most of the assessed loci were monomorphic (Table 4). Furthermore, these populations possessed locus PGI-3 that may have contributed for their divergence from other related wild populations.

Table 6. Estimates of F_{IS} in 13 populations of *Guizotia* at studied loci

LOCUS	C25	G2	F4	B15	U7	W7	J7	B11	E1	A20	B16	I240	B200
AAT-1	-0.5385	-0.3850	-0.250	-0.350	1.000	0.600	-0.125	0.610	0.610	0.524	0.540	....	....
AAT-2	....	-0.1430	-0.053	....	0.614	....	0.600	....	0.250	....	0.608	....	....
AAT-3	1.000	....	....	....	....	....	-0.059	....	1.000	1.000	0.623	....	....
ACP-1	-0.800	1.000	-0.454	-0.778	1.000	1.000	1.000	....	-0.169	....	1.000	-0.158	-0.636
ACP-2	0.410	-0.500	-0.539	-0.518	-1.000	-0.359	-0.414	-0.414	-0.053	-0.540	-0.099	-0.690	-0.690
PGI-1	-0.250	-0.385	-0.111	-0.286	-0.250	-0.540	-0.540	0.380	0.630	-0.540	-0.414	-0.470	-0.570
PGI-2	-0.330	-0.290	0.580	-0.430	0.690	-0.120	-0.290	0.660	1.000	-0.190	0.520	....	....
PGI-3	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	....	....

5.2.2. Genetic diversity between populations

The genetic diversity among the populations was analysed for each polymorphic loci (Table 7). The genic variation based on genotypic frequencies indicated that populations at locus AAT-2 showed the highest diversity followed by AAT-1, ACP-2 and PGI-2. The number of alleles per locus varies in the range of 1 to 5 with a mean of 2.875 (Table 7). In this survey F_{ST} mean value for all populations was estimated as 0.4280 (Table 7) and this indicates that the between populations components account for approximately 43% of very great genetic differentiation among populations in the studied material as suggested by Wright (1978).However, differentiation decrease to 34% when PGI-3 excluded. The AAT-2, AAT-3 and PGI-3 loci contributed particularly great amounts to the observed levels of differentiation among the 13 populations. As *Guizotia* is outcrossing and largely insect-

pollinated, the between differentiation among populations is somewhat higher than with other animal-pollinated outcrossing species (Hamrick and Godt 1989: cited in Park, 2004). The data demonstrates the heterogeneity of the populations within the genus. The genetic divergence is greater in the locus PGI-3 ($F_{ST} = 1$) among the surveyed populations because it is found only in two populations (I_{240} and B_{200}). The extent of inbreeding in the entire populations is about 42%

Table 7. Summary of F-statistics, number of alleles and heterozygosity at studied loci for examined populations of *Guizotia*. Sample size indicates for gametes.

Locus	Sample size	No. Alleles	F_{IS}	F_{IT}	F_{ST}	Nm*	Ho	$H_{exp.}$
AAT-1	232	3.000	0.1571	0.5518	0.4682	0.2839	0.2845	0.5918
AAT-2	232	5.000	0.3974	0.9308	0.8852	0.0324	0.0517	0.7166
AAT-3	234	4.000	0.5512	0.8930	0.7615	0.0783	0.0342	0.4196
ACP-1	234	2.000	0.2597	0.4509	0.2583	0.7180	0.2564	0.4792
ACP-2	258	3.000	-0.4250	-0.2323	0.1353	1.5983	0.6977	0.5656
PGI-1	260	2.000	-0.3603	-0.2730	0.0641	3.6472	0.5154	0.4054
PGI-2	262	3.000	0.2321	0.4410	0.2721	0.6689	0.3282	0.6014
PGI-3	44	1.000	.....	1.0000	1.0000	0.000	0.0000	0.0000
Mean	***220***	***2.8750***	***-0.0068***	***0.4241***	***0.4280***	***0.3341***	***0.5287***	***0.4724***

* Nm = Gene flow estimated from $F_{ST} = 0.25(1 - F_{ST})/ F_{ST}$.

The measure of deviation of genotypic frequency in terms, of hetrozygosity indicated that nearly all the populations' contained equal proportion of heterozygosity and homozygosity ($F_{IS} = 0.1$ when the studied materials is pooled at species level and $F_{IS} = -0.007$ other wise) in the over all loci assessed. This demonstrates that the deviation from Hardy Weinberg expectation becomes insignificant across loci due to opposing forces such as genetic drift, gene flow (migration), mutation and selection acting on different locus brings a balance thus limiting the divergence from Hardy Weinberg Equilibrium.

Table 8. Allelic frequencies of pooled populations at species level of *Guizotia*

Taxa/species	Allele	AAT-1	AAT-2	AAT-3	ACP-1	ACP-2	PGI-1	PGI-2	PGI-3
G. abyssinica	A	-*-----	------	------	0.5000	0.4595	0.8026	0.6579	------
	B	0.6316	------	0.9730	0.5000	0.5000	0.1974	0.3158	------
	C	0.3684	------	0.0270	------	0.0405	------	0.0263	------
	D	------	0.9595	------	------	------	------	------	------
	E	------	0.0405	------	------	------	------	------	------
Chelelu	A	------	------	------	0.2727	0.5000	0.6000	0.1818	------
	B	0.3333	------	0.9000	0.7273	0.5000	0.4000	0.5455	------
	C	0.6667	------	0.1000	------	------	------	0.2727	------
	D	------	0.8636	------	------	------	------	------	------
	E	------	0.1364	------	------	------	------	------	------
G. scabra ssp *schimperi*	A	------	------	------	0.4500	0.3250	0.6500	0.3500	------
	B	0.1389	------	0.9737	0.5500	0.6000	0.3500	0.2750	------
	C	0.8600	0.8750	0.0263	------	0.0750	------	0.3750	------
	D	------	0.1250	------	------	------	------	------	------
G. scabra ssp *scabra*	A	------	------	------	0.8824	0.5789	0.8750	04750	------
	B	0.1500	1.0000	0.6667	0.1176	0.4211	0.1250	0.4500	------
	C	0.8500	------	0.3333	------	------	------	0.0750	------
G. villosa	A	------	------	0.1111	0.7647	------	------	0.0500	------
	B	0.3000	0.9118	0.8611	0.2353	0.6500	0.4000	0.6750	------
	C	0.7000	0.0882	0.0278	------	0.3500	0.6000	0.2750	------
G.arborescens	A	1.0000	1.0000	------	0.7500	0.5909	0.6591	1.0000	1.0000
	B	------	------	------	0.2500	0.4091	0.3409	------	------
	D	------	------	1.0000	------	------	------	------	------

* Nill allele

5.2.3. Allozyme diversity among the Guizotia species

The population pooled at species level of the domesticated crops (*G. abysinica*), Chelelu, *G. scabra* ssp *schimperi* and *G. villosa* showed polymorphism in all the 7 loci where as the other studied species/taxa contained at least one monomorphic locus in the studied enzyme systems. All loci polymorphic at species level share the same common alleles but may possess different ones in low frequencies. The four most diverse loci in both cultivated and

wild species/taxa of *Guizotia* were AAT-1, AAT-2, ACP-2 and PGI-2 with each species having different alleles in high frequency, or unique alleles at one or more of the genes (Table 8).

G. abyssinica has no unique alleles but it contains low frequency of allele C at locus ACP-2 that was not detected in Chelelu. *G. arborescens* contains allozyme A at AAT-1, AAT-2 and allozyme D at AAT-3 with the highest frequency (1.000). These three types of alleles however, were not detected in other wild and cultivated *Guizotia* species and thus may have contributed for their divergence. *G. villosa* exhibits allozyme A at AAT-3 with a frequency of 0.1111, which is unique among the species/taxa (Table 8). Moreover, allele C at ACP-2 occurs with the highest frequency compared to the low frequency in *G. scabra* ssp *schimperi* (0.075) and *G. abyssinica* (0.041), which is unique among them. *G. arborescens, G. abyssinica* and Chelelu are the only taxa containing allele D at high frequency (1.000, 0.9595 & 0.8636 respectively) but are present in low frequency in *G. scabra* ssp *schimperi* (0.125). Unlike Chelelu population, G. *abyssinica* contain allele C at frequency of 0.04 at ACP-2 (Table 8) that may be acquired though introgression.

Table 9. Gene diversity statistics for pooled and partitioned at species/taxa and population level of *Guizotia* respectively. .H_T total gene diversity with in species, Hs = diversity within population, D_{ST} = Gene diversity between population and G_{ST} = degree of differentiation between/among populations.

Species/taxa	A	P	H_O	$H_{exp.}$	H_T	H_S	D_{ST}	G_{ST}
G. abyssinica	2.286	87.50	0.3890	0.3500	0.3661	0.3398	0.2630	0.0718
Chelelu*	2.000	87.50	0.7330	0.4339	0.4339	0.4339	0.000	0.0000
G. scabra ssp *schimperi*	2.286	87.50	0.3500	0.3888	0.3899	0.3766	0.0133	0.0341
G. scabra ssp *scabra*	2.000	75.00	0.1780	0.3207	0.3354	0.2933	0.0421	0.1255
G.villosa	2.286	87.50	0.2880	0.3796	0.3912	0.3660	0.0252	0.0644
G. arborescens	1.375	37.50	0.2500	0.1674	0.1732	0.1689	0.0043	0.0248
Total mean of taxa	***2.039***	***77.08***	***03647***	***0.3401***	***0.3438***	***0.3298***	***0.0185***	***0.0534***

* Chelelu is represented by one population

Total gene diversity (H_T), gene diversity within (Hs) and between (D_{ST}) populations and degree of differentiation between/among populations (G_{ST}) for each taxon are given in Table 9. The total genetic diversity estimates at species/taxa level were considerably variable and range from 0.1732 (*G. arborescens*) to 0.4339 (Chelelu). Chelelu (0.4339), *G. villosa* (0.3912) and *G. scabra* ssp *schimperi* (0.3899) displayed relatively greater gene diversity compared to the other studied species/taxa (Table 9) that could be due to exchange of genetic material between the related taxa/species (Table 2) in addition to other factors that may have contributed for their diversity.

The total mean genetics diversity between populations across all taxa is considerably small ($D_{ST} = 0.0185$) and the same is true of genetics differentiation ($G_{ST} = 0.0534$) for all species whereby only 5% of genetic divergence accounts for between populations of *Guizotia.* This demonstrates that there were higher attributable genetic resemblances of populations in all these taxa/species. Among the wild species of *Guizotias, G. arborescens* had the lowest diversity where only locus ACP-1, ACP-2 and PGI-1 showed polymorphism. The average F-statistics were presented for each species/taxa to see the inbreeding coefficient, the genetic differentiation and gene flow at species levels (Table 10).

Table 10. Mean F-statistics and gene flow of grouped populations at species level

Species	F_{IS}	F_{IT}	F_{ST}	Nm**
G. abyssinica	-0.2072	-0.1206	0.0718	3.2336
Chelelu *	0.3387	0.3387	0.0000	1.0000
G. scabra ssp *schimperi*	0.0429	0.0756	0.0341	7.0768
G. scabra ssp *scabra*	0.3467	0.4287	0.1256	1.7409
G. villosa	0.1703	0.2237	0.0644	3.6306
G. arborescens	-0.5742	-0.5358	0.0244	9.9982
Total mean	**0.1057**	**0.068**	**0.0533**	**4.4463**

* Chelelu represented by single population

**N = population size, m = the average rate of migration

Table 12. Nei's (1978) genetic identity (above diagonal) and genetic distance (below diagonal) between the various populations of *Guizotia* species studied [G. *abyssinica* (pop ID 1-4), Chelelu(5) *G. scabra* ssp *schimperi* (6&7) *G. scabra* ssp *scabra* (8&9) *G. villosa (10&11) G. arborescens* (12&13)].

	Abyssinica				Chelelu	Schimperi		Scabra		Villosa		Arborescens	
pop ID	C_{25}	G_2	F_4	B_{15}	U_7	W_7	J_7	B_{11}	E_1	A_{20}	B_{16}	I_{245}	B_{205}
1	****	0.9595	0.9715	0.9745	0.8960	0.6833	0.7051	0.7026	0.6467	0.5913	0.5974	0.4255	0.4243
2	0.0414	****	0.9291	0.9502	0.9200	0.7007	0.7197	0.6124	0.5637	0.5235	0.6313	0.3659	0.4021
3	0.0289	0.0735	****	0.9902	0.9085	0.7343	0.7383	0.7515	0.6591	0.6969	0.6785	0.4105	0.3956
4	0.0259	0.0511	-0.0039	****	0.9549	0.7488	0.7724	0.7297	0.6385	0.6919	0.7120	0.3779	0.3754
5	0.1092	0.0834	0.0959	0.0462	****	0.7620	0.8485	0.6914	0.6281	0.6599	0.7754	0.2883	0.3143
6	0.3508	0.3557	0.3088	0.2893	0.2710	****	0.9771	0.7315	0.6341	0.6900	0.7775	0.3474	0.3522
7	0.3494	0.3289	0.3034	0.2583	0.1643	0.0232	****	0.7266	0.6602	0.6812	0.7915	0.3089	0.3223
8	0.3529	0.4904	0.2858	0.3152	0.3691	0.3127	0.3193	****	0.9011	0.9287	0.8339	0.4125	0.3737
9	0.4358	0.5732	0.4168	0.4487	0.4651	0.4556	0.4152	0.1041	****	0.8162	0.7807	0.4041	0.3859
10	0.5254	0.6472	0.3612	0.3683	0.4159	0.3582	0.3839	0.0739	0.2031	****	0.9363	0.3099	0.2683
11	0.5151	0.4601	0.3878	0.3389	0.2544	0.2516	0.2338	0.1816	0.2476	0.0659	****	0.2324	0.2433
12	0.8545	1.0053	0.8903	0.9730	1.2439	1.0572	1.1749	0.8855	0.9060	1.1749	1.4595	****	0.9950
13	0.8572	0.9110	0.9268	0.9797	1.1573	1.0435	1.1323	0.9844	0.9526	1.3167	1.4134	0.0050	****

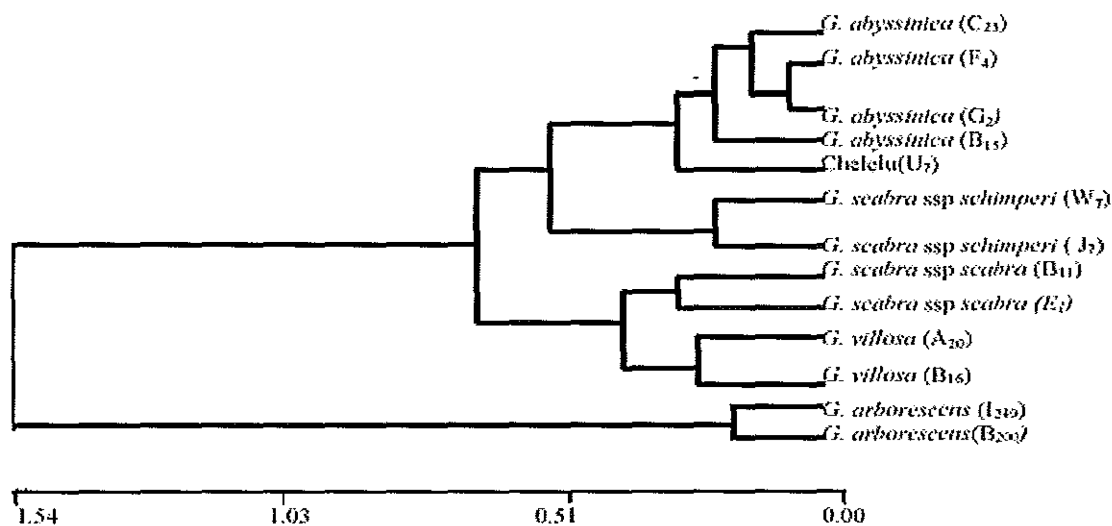


Figure 4. UPGMA phenogram derived from Nei's (1978) genetic distance among 13 Populations (in parethesis) of six *Guizotia* species/taxa.

Dendrograms were constructed using a matrix of genetic distance (Nei, 1978) from pairwise comparisons of populations of all species/taxa at two hierarchies and is presented in Figure 4 and 5. They were constructed using the UPGMA. All the populations in each taxon appeared to be clustered together. UPGMA analysis leads to three main clusters. The first cluster includes both domesticated and wild populations of *Guizotia* (F_4, G_2, B_{15}, C_{25}, U_7, W_7 and J_7). This cluster displayed two sub clusters, which were morphologically close to each others (Baagoe, 1974 and Kifle Dagne, 1994). The second cluster contained *G. scabra* ssp *scabra* (population B_{11} and E_1) and *G. villosa* (population A_{20} and B_{16}). The third cluster group holds *G. arborescens* (population I_{240} and B_{200}), which were distant from any of the studied wild and domesticated *Guizotias*. The same result was produced when all populations were pooled at species level (Figure 5).

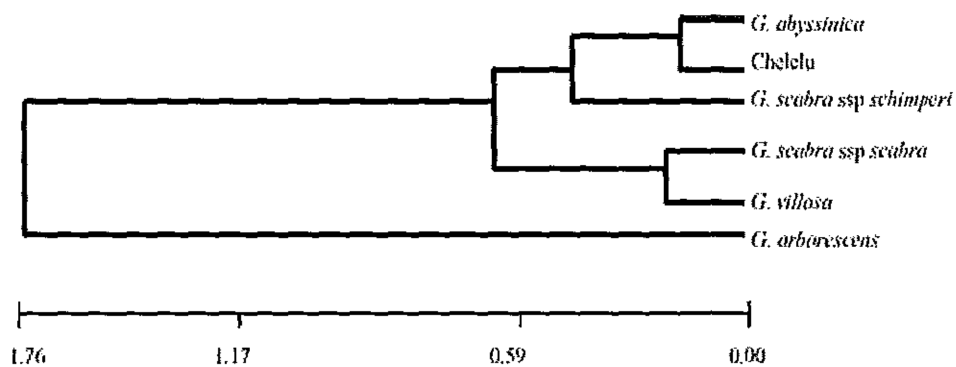
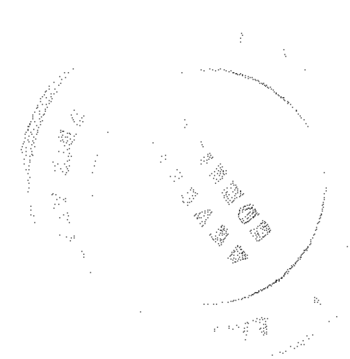


Figure 5. Dendrogram derived from genetic distance of pooled populations at species level



6. Discussion

Little data is available in the literature on the genus *Guizotia* related to isozyme analysis so far and thus we were forced to use most related genus/families or species to *Guizotia* for interpretation of the isozymes studied (Doebley, 1989). Cronn *et al.* (1997) reported three AAT zones with three to four allozymes at each locus. Carerra *et al.* (2002) reported two zones of ACP and three zones PGI in most closely related species (sunflower) almost compatible with our results. Although the genes, which code isozymes in different species, are conservative (Gottleib, 1977), some degree of differentiation may occur in certain zones. Change in the DNA of an enzyme locus may change the amino acids, which are in a sequence, and these may change the amount of static charge on an enzyme molecule (Kephart, 1990; Wiley and Sons, 1990; Murthy *et al.*, 1996). As a result of this difference in migration rate between different species are due to some degree of genetic variation among the taxa.

6.1. Genetic variation within population

The genetic variation in this study was high both at populations and species level compared to the result of closely related species reported (Crawford *et al,* 1984; Cronn *et al,* 1997; Carerra *et al.*, 2002). *Guizotia* being cross-pollinated plants, the value for alleles per locus at the species and populations level observed were nearly similar (A = 1.871 at population level; A = 2.03 at species level) with the same breeding system (Hamrick and Godt, 1989:cited in Park, 2004). However, the utilization of a number of alleles per locus as a measure of genetic variability among populations may cause sampling errors due to variation in population size (Nei, 1987).

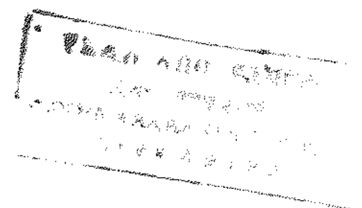
The value of percentage of polymorphic loci (mean = 77%) and gene diversity were also high with in the populations (H_{exp} = 0.3233) and at species level (H_{exp} = 0.3401) unlike that of the most closely related species/genus like *Helianthus species* (A = 2.21, P = 78%, H_{exp} = 0.2467) (Cronn *et al.*, 1997). Such greater genetic diversity could be the result of the out crossing nature of the genus, the different selection pressure acting on different genotype in favour or disfavour of hetrozygote in different geographic regions and its higher

polymorphic nature (all the studied loci polymorphic in range of 37.5% to 87.5%). Unlike all populations surveyed, population I_{240} and B_{200} showed relatively low genetic variability at populations level (H_{exp} = 0.1689) and at species level (0.1674). Such significant low variability might be explained by both populations taken from adjacent geographical regions that favour free flow of gene between populations (Omo nada and Chida of Jimma) and/or the consequent bottleneck effect (Park, 2004).

Since the genus *Guizotia* is self-incompatible significant deviation from Hardy Weinberg expectations is expected. The Chi-square test (X^2) revealed that nearly 88% of populations in the common polymorphic loci were deviated from Hardy Weinberg proportions. And 9% of these populations were fixed with alternative alleles when the populations were analysed separately and grouped at species level. Such deviations were probably due to small population size, non-random mating or/and massive migration from a genotypically different population.

The fixation index (F_{IS}) is useful for understanding the breeding structure of populations or the pattern of selection associated with polymorphic loci. F_{IS} value of 29% of populations lie between 0.25 and 1.00 indicating that non-random mating and presence of null alleles or both may result in deficiency of heterozygosity (Table 6). On the other hand nearly 19% of the populations displayed balanced heterozygosity possibly due to counterbalancing forces (e.g. gene flow, genetic drift, selection vs mutation, and recombination) in favour of different genotypes acting at those particular loci in both cultivated and wild populations. Nearly 47% of the population had excess heterozygosity (F_{IS}<0) indicating out breeding nature and accumulation of variation through mutations and recombination within the populations or selection acting in favour of hetrozygote in different geographical distribution of the populations.

According to Nei's (1978) unbiased measure of genetic diversity, considerable genetic variances were recorded within the populations in the range of 0.151 (I_{240}) to 0.4339 (U_7). Among the cultivated populations, population B_{15} (from Gonder) showed high genetic diversity ($H_{exp.}$ = 0.3682) while from wild populations, population U_7 (0.4339) and population B_{16} (0.4227) displayed high genetic variation. Such variation could be explained by the effect of environmental variation, change in geographical continuity and soil factors



that contribute for gene diversity with in populations (Park, 2004).

6.2. Genetic variation between populations

The genetic diversity level should be interpreted in the context of the interaction among different factors influencing populations' dynamics such as mating system, mutation, gene flow and environmental conditions (Nei, 1987). The highest level of variations at a locus AAT-1, AAT-2, ACP-2 and PGI-2 were probably due to high allelic richness, mutation and absence of negative selection pressure acting on these enzymes at these particular loci in wide range of their geographical distribution (Nei, 1987). The mean F_{ST} from all population of surveyed loci value (0.4280) indicate genetic differentiations that are displayed among the populations. According to Wright (1978) criteria there was nearly 43% of the variation detected among the populations (Table 7). This result is consistent with the hypothesis that the taxa share a recent common ancestry and that there is substantial ongoing gene flow between species regardless of their heterogenity.

A higher genetic divergence exists at a locus AAT-2, AAT-3, and PGI-3 with the F_{ST} value of 0.885, 0.762 and 1.000 respectively among the populations that contributes for greater genetic differentiation. The occurrence of a locus PGI-3 only in populations B_{200} and I_{240} may be due to mutation or introgression around its distributions (Table 8). The gene flow becomes maximum at PGI-1 (3.647) and minimum at PGI-3 (0.000) indicating various rate of selection or mutation acting on migrating alleles among the populations at different loci.

Generally, outbreeders commonly have the highest level of allozyme variation within populations whereas inbreeders have the highest variation between populations (Hamrick and Godt 1989: cited in Park, 2004) in concordant with our result of F_{ST}-parameter .The over all average measures of deviation of genotypic frequency within the eight loci across populations in terms of deficiency or excess heterozygosity (F_{IS} = -0.007) indicate that the number of hetrozygote and homozygote are nearly balanced (Table 7). This result demonstrates that probably selection pressure acting on populations in favour or disfavour of hetrozygote in different regions on different loci result in balanced hetrozygote among the populations.

6.3. Allozyme diversity within and among the *Guizotia* species

Large ranges of proportion of polymorphic loci are exhibited in species/taxa *G. scabra* ssp *schimperi, G. villosa and G. scabra* ssp *scabra* (Table 5). When populations are pooled at a species level only, *G. arborescens* had the lowest values of percentage polymorphic loci (37.5 %). Populations of all closely related species except *G. arborescens* exhibited comparable values for average numbers of alleles per locus (Table 5). These two measures of genetic variation indicate populations of all species are of similar level of variation. Chelelu showed greatest genetic variability unlike all the other populations. High genetic diversity in rare species/taxa is usually associated with the unique history of the species/taxa such as its recent origin from a widespread ancestor, multiple origin, hybridization, or ecological traits such as the ability to survive in marsh habitats unlike other related taxa (Godt and Hamrick, 1998; Park, 2004; Gitzendanner and Soltis, 2000). On the other hand, the low genetic variation in *G. arborescens* might be selection in favour of homozygote in AAT loci, AAT-1, 2,3, in PGI loci, PGI-2, 3 that contributed to fixation of alternate alleles. Alternately genetic drift and bottleneck effect may have reduced the genetic variation of the species (Ayres and Ryan, 1999; Godt and Hamrick, 1998).

All of the populations grouped at species level deviated from the expectation of Hardy Weinberg equilibrium (Table 10). Any deviation from expected heterozygosity for a given species is either excesses (*G. arborescens* and *G. abyssinica*) or deficiencies (*G. scabra* ssp *scabra, G. villosa, G. scabra* ssp *schimperi* and Chelelu) of heterozygote. Our observation of homozygote deficiency in these taxa could be due to high out crossing rates or mating among genetically different individuals in large populations or because gene flow introduces variation into populations. Deficiencies of heterozygosity from expected value might be due to selection that disfavour hetrozygote or due to the sub division of populations into many breeding units (Wahlund effect) (Nei, 1987).

When the populations of all taxa are considered in-groups at species level (Table 9), the mean gene diversities within populations (Hs = 0.3298) in all taxa and the between populations (D_{ST} = 0.0185) were significantly different demonstrating the out breeding nature of populations within species/taxa (Gitzendanner and Soltis, 2000; Park, 2004). The value of G_{ST} (0.0534) in all taxa indicated that only 5% of the genetic variation in all species is due to between

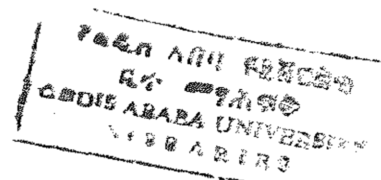
is due to between populations gene differences signifying that greater genetic diversity lies within populations than between populations in all taxa/species. Chelelu population had the greatest amount of gene diversity (H_T = 0.4339) in conflict to the concept that widely distributed populations and widespread species maintain more allozyme variation than restricted species (Gitzendanner and Soltis, 2000). On the other hand a relatively less geographically restricted species, *G. arborescens*, had the least genetic variation (H_T= 0.1732). Such low genetic variability may be due to high rate of gene flow between adjacent populations and the genetic drift that probably cause fixation of common alleles and loss of rare alleles (Nei, 1987).

However, recent comparative studies of genetic variation between rare and widespread species have demonstrated that several rare or endemic species are polymorphic as their widespread congeners (Godt and Hamrick, 1998). Thus, it is difficult to state that species with limited geographic range always have low genetic diversity. Accordingly, these data established that the majority of genic diversity in the species/ taxa resides within (H_S) populations. This is an important feature in the studied material because genetic diversity within the species is necessary for the growth and improvement of crops in diverse environments (Endashaw Bekele, 1985). Relatively higher amount of between populations' differentiations was seen in *G. scabra* ssp *scabra* (G_{ST} = 0.1255). The possible reason is that there was greater geographic distance between the two populations (Tigray and Sidamo) and consequently reproductive isolations that is relatively higher increase the between populations genetic differtiation to a considerable amount.

The genetic diversity in populations of the individual species is greater than that which has been reported in other outcrossing plant of closely related genus *Antennaria* (Bayer and Crawford, 1986) and *Coreopsis* (Crawford and Bayer, 1981). Such results might be due to: (1) small sample size of the population assessed per species/taxa (2) small number of enzymes assayed or/and (3) selection pressure acting on each population in each species on different geographical location differently and allelic richness within the Genus. Generally the existence of high genetic diversity within populations, little differentiation among populations and high levels of gene flow indicate that these species are predominately out crossing.

The genetic identities for pair wise comparisons of populations within each species range from 0.9011 (*G. scabra* ssp *scabra*) to 0.995 (*G. arborescens*) (Table 12) indicating essentially no interpopulation differentiation within species. Similarly high identities have been reported for the vast majority of species examined electrophoretically (Gottlieb, 1977, Gottlieb, 1981: cited in Wiley and Sons, 1990). The gene identities for populations of different species revealed that *G. abysinica* and Chelelu are the most similar (I = 0.9306) which are partly in agreement with the morphological and cytological data ((Kifle Dagne, 1994; 1995). *G. abyssinica* was less similar to *G. arborescens* (I = 0.4039) in which the same result was obtained in chromosomal studies (Kifle Dagne, 1995) (Table 11).

Relatively higher genetic identity was observed between *G. abyssinica* & *G. scabra* ssp *schimperi* (I = 0.7531), Chelelu & *G. scabra* ssp *scabra* (I = 0.7136), and Chelelu & *G. scabra* ssp *schimperi* (I = 0.8245) signifying close similarities among these taxa in support of the result of meiotic behaviours and pollen fertility (Kifle Dagne, 1994, 2001a). Morphologically, *G. scabra* ssp *schimperi*, *G. abyssinica* and Chelelu are most similar (Kifle Dagne, 1994) demonstrating concordance between electrophoretic and morphological data. *G. arborescens* showed distant relationship to the most closely related groups of species/taxa (Chelelu, G. *abyssinica* & *G. scabra* ssp *schimperi*) with the average genetic distance of 1.066 next to the divergence between *G. arborescens* and *G. villosa* (D = 1.2955) (Table 11) thus signifying long time of divergence among these species/taxa.



6.4. Phenetic Analysis

A cluster analysis (UPGMA) of the inter population genetic distance matrix summarized the different hierarchal data sets (Figure 4 and 5) and showed groupings more or less similar to that of the cytological and morphological data. Constructed dendrogrames based on the different isozyme data sets [within each and pooled populations at species level (Figure 4 & 5)] displayed certain similarities. Comparison of the two dendrograms (Figure 4,5) showed that *G. arborescens* (I_{240} & B_{200}) was rather isolated and all contain three clusters regardless of the different isozyme data employed.

The first cluster contains *G. abyssinica* (G_2, C_{25}, F_4 and B_{15}), Chelelu (U_7) and *G. scabra* ssp *schimperi* (J_7, W_7) that had a close morphological and cytological resemblance with the

cultivated crops (Kifle Dagne, 1994, 1995, 2001a.). This may be due to common alleles they shared in their evolutionary history. Populations of *G. scabra* ssp *scabra* (B_{11} & E_1) and *G. villosa* (A_{20}, B_{16}) (D = 0.1266) are grouped into the second cluster and are in accordance with the karyotype, seed set and chromosomal pairing studies (Hiremath *et al*, 1992; Kifle Dagne, 1994,1995). The species *G. arborescens* widely separated from all other studied species /taxa (Figure 4 & 5) with long divergence time, which is in agreement with chromosomal pairing, hybridisation and karyotypic studies (Kifle Dagne, 1994, 1995, 2001a). Thus the dendrograms generated based on each population and pooled at species level (Figure 4 & 5) were nearly congruent to each other.

6.5. Phylogenetic relationships

The phylogenetic relationships between wild and cultivated populations referred to in this study are, by and large, consistent with morphological and cytological studies (Hiremath and Murthy, 1988; Kifle Dagne, 1994, 1995, 2001a). The result obtained in this study agrees with the suggestion of Doebley (1989) regarding wild-cultivated derivatives. When isozymes are analysed: (1) the cultigens falls within the variation range of the putative wild progenitors (2) the cultigens have a subset of the allelic diversity found in the wild progenitors and (3) the cultigens have less genetic variation than the wild population. Crops with their possible progenitors share a very close allozymic relationships (Doebley, 1989; Wiley and Sons, 1990). High genetic identity between Chelelu and the domesticated species, *G. abyssinica* (I = 0.9306), showed that Chelelu is the most possible progenitor of the domesticated species *G. abyssinica* partly in support of the chromosomal pairing, meiotic behaviours and pollen fertility studies by Kifle Dagne (1995,2001a) (Table 2 and 11).

Other close relationships were found between *G. scabra* ssp *scabra* & *G. villosa* (in accordance with chromosomal studies by Kifle Dagne, 1995), and *G. scabra* ssp *schimperi* & *G. villosa* with the genetic distance of 0.1266 and 0.2795, respectively (Table 11). The genetic distance between *G. scabra* ssp *scabra* and *G. scabra* ssp *schimperi* (0.3374) is higher than, between *G. villosa* and *G. scabra* ssp *scabra,* signifying relatively greater genetic divergence between these subspecies.

However, 33 out of 42 alleles were common to all populations except I_{240} and B_{200} [at species/taxa level, 15 alleles out of 20 are common to all species/taxa except *G. arborescens*] confirming that they acquired such alleles either through introgression (Jencewski *et al.*, 2003) or shared a common evolutionary history (Doebley, 1989) which are in line with the result of chromosomal studies of *Guizotia* (Kifle Dagne, 1994, 1995, 2001a).

The morphological and cytological investigations suggested that *G. scabra* ssp *schimperi* were the possible progenitors of the cultigens (*G. abyssinica*) (Hiremath and Murthy, 1988; Baagoe, 1974). Nevertheless, the result of isozyme data was not strong enough to confirm that Chelelu is the only ancestor for the cultivated crops because of the limitation such as population size of Chelelu used in the study, the number of enzyme systems assessed and even the limitation of marker we used for phylogenetic studies (Willey and Sons, 1990; Doebley, 1989). Besides, to this, the number of alleles, proportion of polymorphic loci and the gene diversity could not discriminate undoubtedly Chelelu, *G. scabra* ssp *schimperi* and *G. abyssinica.* Further more, the isozyme variation among Chelelu, *G. scabra* ssp *schimperi* and *G. abyssinica* in terms of genetic distance, indicated that they were closely related to each other in the range of 0.0719-0.284 in agreement with studies of chromosomal pairing and morphology, pollen fertility and meiotic behaviours (Kifle Dagne, 1994, 1995, 2001a) (Table 11).

In spite of the result of isozyme data, the poor crossability between Chelelu and *G.abyssinica*, morphological differences (e.g. perennial vs annual habits), differences in number of florets and geographic distribution, contributes for the doubtful progenitor-derivative relationship between Chelelu and *G. abyssinica.* Like wise, the formation of hybrid plants between this population and distantly related taxa of the genus, *G. zavattarii,* demonstrate the need of further studies between wild taxa and the cultivated species.

Due to fact that the genus *Guizotia* contains groups closely related taxa it is not easy to determine clearly phylogenetic relationships from previous studies of hybridisations, chromosomal pairing, karyotype analysis as well as the present isozyme data. Hence additional data from biochemical, molecular studies are important in elucidating the phylogenetic relationships between the various taxa of the genus.

7. Conclusions and Recommendation

7. 1. Conclusions

- The result of the present study shows that there is concordance between morphological, cytological and enzyme divergence among the studied taxa (e.g. *G. abyssinica* and *G. scabra* ssp *scabra; G. villosa* and *G. arborescens*). Taxa that appeared most dissimilar and showed lower genetic identities may be the result of longer isolation time with progressive levels of divergence in morphology and isozymes.

- Mean genetic identities among/between populations of each taxa are quiet high, often above 0.9, which is in agreement with that of other out-crossing plants, where most populations contain the same high frequency alleles.

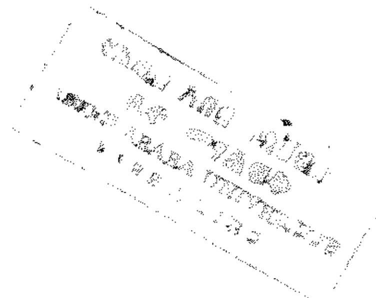
- The phenetic analysis based on genetic distance among populations/taxa provided insights into phylogenetic relationships among species/taxa. In our study, *G. abssyinica* and Chelelu show high genetic similarity compared to the other studied taxa. Hence, Chelelu become the possible progenitor of the cultivated *G. abyssinica.*

- The genetic divergence between closely related group of taxa (*G. abyssinica,* Chelelu, *G. scabra* ssp *schimperi* etc.) and *G. arborescens* from our result was in agreement with the morphological and cytological studies.

7. 2. Recommendations

- The study of genetic variation within or among taxa (the evolutionary relationship), between *G. abyssinica* and its wild relatives so far indicate close relationships among taxa. Thus, further study using alternative genetic markers is mandatory to find out desirable traits that help to increase the yield and quality of the crops, which is to date the main agronomic problem in the country.

- The researcher, plant breeders and government must give due attention to obtain better yields and better quality varieties of this crops through crops improvement programs as alternative means of assisting food securities because of its higher demand in domestic and world market.



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