Genet Resour Crop Evol (2008) 55:451–458
DOI 10.1007/s10722-007-9251-4

RESEARCH ARTICLE

Analysis of genetic diversity and relationships of wild *Guizotia* species from Ethiopia using ISSR markers

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Received: 6 January 2007 / Accepted: 7 May 2007 / Published online: 14 July 2007

Abstract Genetic relationships and diversity of 45 *Guizotia* populations each consisting of ten individuals and belonging to five taxa of the genus *Guizotia* were analyzed using Inter Simple Sequence Repeat (ISSR) markers. Five ISSR primers generated a total of 145 scorable bands across the 450 individuals used for the study. The percent polymorphic loci for the taxa ranged from 68.2 (*G. arborescens*) to 88% (*G. scabra* ssp. *schimperi*), with *G. scabra* ssp. *scabra*, *G. zavattarii* and *G. villosa* following *G. scabra* ssp. *schimperi* in this order with respect to the abundance of percent polymorphic loci. The Shannon-Weaver diversity indices (H′), for the five taxa also followed a similar pattern, with *G. scabra* ssp. *schimperi* exhibiting the highest H′ (0.7373) and *G. arborescens* the least (0.5791), while H′ for *G. scabra* ssp. *scabra*, *G. villosa* and *G. zavattarii* were 0.7313, 0.6620 and 0.6564, respectively. The least genetic distance (0.1188) was observed between *G. scabra* ssp. *schimperi* and *G.villosa*, revealing closer genetic relationships of the two species with each other than with the others, and the highest genetic distance (0.2740) was observed between *G. scabra* ssp. *schimperi* and *G. zavattarii*. The unweighted pair group method using the arithmetic average clustering of the five taxa using the standard genetic distances produced two clusters, with *G. scabra* ssp. *schimperi* and *G. villosa* occurring in one cluster and *G. scabra* ssp. *scabra*, *G. arborescens* and *G. zavattarii* together in the other cluster. The study reveals that *G. scabra* ssp. *schimperi* is more closely related to *G. villosa* than to *G. scabra* ssp. *scabra*.

Keywords Gene flow · Genetic diversity · *Guizotia* · Polymorphic loci · UPGMA

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Introduction

The genus *Guizotia* belongs to the family Asteraceae (Compositae), tribe Heliantheae, subtribe Coreopsidinae. The taxonomic revision of the genus was made by Baagoe (1974) who reduced the number of species to six. The genus *Guizotia* consists of six species and two subspecies five of which are found in Ethiopia (Baagoe 1974). These are *G. abyssinica* (L.f.) Cass., *G. scabra* (Vis.) Chiov. ssp. s*cabra; G. scabra* (Vis.) Chiov. ssp. *schimperi* (Sch. Bip.) Baagoe; *G. villosa* Sch. Bip., *G. zavattarii* Lanza;

Guizotia arborescens (I. Friis) and *G. jacksonii* (S. Moore) J. Baagoe. All the six species grow in East Africa. The chromosome number of all *Guizotia* species as reported by Dagne (1994a) is $2x = 2n = 30$.

The distribution of the *Guizotia* species in Africa and in Ethiopia varies greatly. Some of the species

such as *G. villosa*, *G. arborescens*, *G. zavattarii* and *G. jacksonii* are restricted in their distribution, while others like *G. scabra* ssp. *scabra*, *G. scabra* ssp. *schimperi* and *G. abyssinica* cover a relatively wide area in east Africa with a greater concentration in Ethiopia. *G. arborescens* is a component of the natural vegetation in the Imatong mountains of Uganda and Sudan and southern Ethiopia (Friis 1971; Baagoe 1974; Hiremath and Murthy 1988). *G. zavattarii* is endemic to mount Mega in southern Ethiopia and the Huri hills in northern Kenya (Dagne 1994a; Baagoe 1974; Hiremath and Murthy 1988). The distribution of *G. scabra* ssp. *scabra* covers a wide range extending from east Africa to Nigeria with a distributional gap in the rain forest of Congo, while *G. scabra* ssp. *schimperi* is a common weed of cultivated crops and widely distributed in Ethiopia (Hiremath and Murthy 1988; Dagne 1994a). *G. villosa* is endemic to the northern part of Ethiopia and *G. jacksonii* is endemic to mount Kenya, Aberdares and Mt. Elgon in Uganda and Kenya (Dagne 1994a; Hiremath and Murthy 1988).

Murthy et al. (1995) tried to revise the present taxonomic classification of the genus that was done by Baagoe (1974). Baagoe's classification of the genus was based on morphological similarity. Murthy et al. (1995) concluded that classifying *G. scabra* ssp. *schimperi* as a subspecies of *G. scabra* was not sound on the basis of their karyomorphological studies. While the existing classification of the genus is based on morphological similarities, karyomorphological studies, however, reveal that *G. scabra* ssp. *schimperi* is more closely related to *G. abyssinica* than to *G. scabra* ssp. *scabra* (Dagne 1994b; Murthy et al. 1993, 1995). Similar conclusion was made by Hiremath and Murthy (1992) who suggested that *G. abyssinica*, *G. scabra* ssp. *schimperi* and *G. villosa* are more closely related as they have symmetrical karyotypes unlike *G. zavattarii*, *G. scabra* ssp. *scabra* and *G. jacksonii* which have asymmetrical karyotypes. Furthermore, Dagne (1994b) showed that *G. scabra ssp. schimperi*, *G. scabra* ssp. *scabra*, *G. villosa* and *G. abyssinica* are closely related because of the high level of crossability among them. His studies revealed that chromosome homology is higher between *G. scabra* ssp. *schimperi* and *G. abyssinica* than between any other two taxa he studied. The controversy, however, on the taxonomic status of *G. scabra* ssp. *schimperi* still remains.

However, to date little work has been done on the genetic relationships of the *Guizotia* species. In fact no work has been done on the genetic diversity and relationships of the *Guizotia* species using ISSR markers. The utility of ISSR to study genetic relationships and identify taxa within a genus has been shown by several workers (Ajebade et al. 2000; Rajesh et al. 2002; Tikunov et al. 2003; Vijayan and Chatterjee 2003; Sudupak 2004). ISSR is a new approach of direct DNA analysis developed in 1994 (Zietkiewicz et al. 1994). The technique relies on the fact that eukaryotic genomes contain an abundance of Simple Sequence Repeats (SSR) (Lagercrantz et al. 1993). The SSR sequences are used as primers in polymerase chain reactions (PCR) to amplify regions between SSR loci (Zietkiewicz et al. 1994).

Inter Simple Sequence Repeat analysis of *G. abyssinica* populations collected from eight niger growing regions of Ethiopia was done by Petros et al. (2007). It was shown that the technique reveals high polymorphism within and among the niger populations of Ethiopia. In fact the technique was able to discriminate between the different strains of niger that were identified based on the duration to flowering and maturity. Geleta et al. (2007) also studied the genetic diversity of niger populations from Ethiopia as revealed by random amplified polymorphic DNA and reported a high degree of polymorphism among and within the populations. As *G. abyssinica* is the most important oil crop in Ethiopia, the present work becomes worthwhile because attempts to improve its agronomic or oil quality, whether it be through conventional plant breeding or genetic engineering would, to a certain extent rely on the knowledge of its wild relatives. The present work is therefore an attempt to study the genetic diversity and relationships among five of the *Guizotia* taxa growing in Ethiopia using ISSR markers envisaging that the information obtained from this investigation might be used by future investigators in their endeavor to improve the agronomic quality of niger.

Materials and methods

The plant material and DNA extraction

The plant materials used in the study were collected during November–December 2003. The materials

include 27 populations of *G. scabra* ssp. *schimperi* collected from 11 regions in the country (Table 1). Eight populations of *G. villosa* collected from Gojam and Gonder, seven populations of *G. scabra* ssp. *scabra* from Jimma, Illubabor and Wellega, two populations of *G. zavattarii* from Sidamo and one population of *G. arborescens* from Kaffa. While every effort was made to include all the wild *Guizotia* taxa growing in Ethiopia in the study, not all were, however equally represented in the collection. This anomaly stems from the fact that some of the species such as *G. villosa*, *G. zavattarii* and *G. arborescens* are highly restricted in their distribution while others like *G. scabra* ssp. *scimperi* are found growing all over the country. Thus, 27 populations of *G. scabra* ssp. *schimperi* were collected while *G. scabra* ssp. *scabra* was represented by seven populations from three of the western regions of the country where they occur. *G. villosa*, *G. zavattarii* and *G. arborescens* on the other hand were very much localized in their distribution. *G. villosa* grows only in certain areas in Gojam, Gonder and Tigray in the north and collection was made from Gojam and Gonder. *G. zavattarii* grows in very restricted areas covering small areas near Yabello town and Mt. Mega in the south. As this species has a continuous distribution over a small area, the authors considered the Yabello material as a single population and that from Mt. Mega as another. *G. arborescens* on the other hand, has the most restricted distribution of them all, growing near Omo Nada in the south west. In fact all the *G. arborescens* materials used by Friis (1971) to describe the species came from this area around Mount Maigudo. Thus, the inclusion of only a single population of *G. arborescens* and only two populations of *G. zavattarii* is due to their highly localized distribution.

The achenes (seeds) collected from Ethiopia were grown in a greenhouse at the department of crop science of the Swedish University of Agricultural Sciences (SLU).

The plant genomic DNA was extracted following the CTAB (cetyltrimethyl ammonium bromide) method as applied by Assefa et al. (2003).

PCR amplification and electrophoresis

Polymerase chain reaction was performed by means of five ISSR primers that were selected out of 15 tested (Table 2). The primer selection was based on the degree of polymorphism and the distinctness of the bands they produced when tested on a sample set. The PCR reaction mix was a 25 µl volume containing 10 ng of genomic DNA, 1× PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl), 2 mM $MgCl_2$, 0.2 mM of the dNTP's (dATP, dCTP, dGTP, dTTP), 2% Formamide, 0.2 µM primer, 0.05 U/µl of Taq DNA polymerase and deionized water to make up the reaction volume. Amplification of DNA was performed in a GENE AMP PCR thermocycler (HITACHI Ltd, Tokyo, Japan), programmed for the following temperature profiles: 1 min of initial denaturation at 94°C followed by 40 cycles, each consisting of a denaturation step at 94°C for 1 min, an annealing step at 55°C for 2 min, and an extension step at 72°C for 2 min, with a final extension at the end of the 40 cycles at 72°C for 5 min. Products were electrophoresed in polyacrylamide gels supplied by Amersham Pharmacia Biotech AB, along with two lanes of size markers. Fragments were visualized by silver staining on the Hoefer Automated gel stainer (Pharmacia Biotech). DNA fragment sizes were estimated by comparing the DNA bands with a 100 base pair ladder marker loaded in the peripheral wells of the gel on either side of the sample wells.

Data analysis

The bands were recorded as present (1) or absent (0), and assembled in a data matrix. POPGENE software 1.31 (Yeh et al. 1999) was utilized to generate the single population gene frequencies and the grouped population gene frequencies as well as Nei's (1972) genetic distances matrix between the populations from the 0, 1, data matrix. The resulting single population gene frequencies were used to construct an unweighted pair group method using the arithmetic average (UPGMA) phenogram for the populations using a software package, Genetic Distances and Phylogenetic Analysis (DISPAN 1993). DISPAN (1993) was also used for the analysis of the Grouped population gene frequencies to generate the standard genetic distances matrix between the five *Guizotia* taxa studied and the resulting distance matrix used to construct an (UPGMA) phenogram for the five taxa. Nei's (1973) genetic diversity parameters; the total genetic diversity (Ht), the within population genetic diversity (Hs), the among population genetic diversity (Dst) and the coefficient of genetic differentiation

Table 1 Region of collection, altitude, site coordinates (description) of the collection sites of the Guizotia species used in the study

No	Name	Region	Pop. code	Site coordinates	Altitude (m)
1	*Guizotia scabra* ssp. *schimperi*	Hararghe	gsp1	09°01′01N, 040°53′47E	2,384
2	*Guizotia scabra* ssp. *schimperi*	Hararghe	gsp2	09°58′06N, 040°50′17E	2,147
3	*Guizotia scabra* ssp. *schimperi*	W. Shewa	gsp3	08°38′25N, 038°09′35E	2,292
4	*Guizotia scabra* ssp. *schimperi*	W. Shewa	gsp4	08°45′43N, 038°18′20E	2,114
5	*Guizotia scabra* ssp. *schimperi*	W.Shewa	gsp5	08°42′10N, 038°15′04E	2,153
6	*Guizotia scabra* ssp. *schimperi*	N. Shewa	gsp6	09°45′09N, 038°28′13E	2,283
7	*Guizotia scabra* ssp. *schimperi*	Jimma	gsp7	08°09′17N, 037°31′43E	1,728
8	*Guizotia scabra* ssp. *schimperi*	Jimma	gsp8	08°03′39N, 037°22′10E	1,891
9	*Guizotia scabra* ssp. *schimperi*	Illubabor	gsp9	08°22′23N, 036°12′38E	2,167
10	*Guizotia scabra* ssp. *schimperi*	Illubabor	gsp10	08°23′14N, 035°59′10E	1,618
11	*Guizotia scabra* ssp. *schimperi*	Wellega	gsp11	09°02′56N, 036°23′50E	2,007
12	*Guizotia scabra* ssp. *schimperi*	Wellega	gsp12	09°01′43N, 036°38′11E	2,245
13	*Guizotia scabra* ssp. *schimperi*	Arsi	gsp13	07°49′08N, 039°08′31E	2,276
14	*Guizotia scabra* ssp. *schimperi*	Arsi	gsp14	07°13′10N, 039°14′31E	2,517
15	*Guizotia scabra* ssp. *schimperi*	Bale	gsp15	07°12′47N, 040°30′48E	2,177
16	*Guizotia scabra* ssp. *schimperi*	Bale	gsp16	07°00′18N, 039°28556E	2,411
17	*Guizotia scabra* ssp. *schimperi*	Gojam	gsp17	10°21′15N, 037°12′12E	2,394
18	*Guizotia scabra* ssp. *schimperi*	Gojam	gsp18	10°22′16N, 037°36′24E	2,249
19	*Guizotia scabra* ssp. *schimperi*	Gojam	gsp19	10°31′25N, 037°31′17E	2,097
20	*Guizotia scabra* ssp. *schimperi*	Gojam	gsp20	10°41′32N, 037°12′31E	2,006
21	*Guizotia scabra* ssp. *schimperi*	Gonder	gsp21	12°39′16N, 037°29′14E	2,246
22	*Guizotia scabra* ssp. *schimperi*	Gonder	gsp22	12°48′11N, 037°19′19E	2,682
23	*Guizotia scabra* ssp. *schimperi*	Gonder	gsp23	12°40′27N, 037°30′10E	2,429
24	*Guizotia scabra* ssp. *schimperi*	Gonder	gsp24	12°43′14N, 037°29′30E	2,690
25	*Guizotia scabra* ssp. *schimperi*	Well	gsp25	11°43′06N, 038°55′08E	2,522
26	*Guizotia scabra* ssp. *schimperi*	Well	gsp26	11°43′50N, 038°55′32E	2,375
27	*Guizotia scabra* ssp. *schimperi*	Sidamo	gsp27	06°04′27N, 038°13′27E	2,328
28	*Guizotia villosa*	Gojam	Gv28	11°27′01N, 037°23′57E	1,922
29	*Guizotia villosa*	Gojam	Gv29	11°22′37N, 037°24′38E	2,220
30	*Guizotia villosa*	Gojam	gv30	11°17′10N, 037°28′49E	2,210
31	*Guizotia villosa*	Gojam	gv31	11°21′54N, 037°25′19E	2,296
32	*Guizotia villosa*	Gojam	gv32	11°17′28N, 037°2834E	2,247
33	*Guizotia villosa*	Gonder	gv33	11°53’01N, 037°39′54E	1,825
34	*Guizotia villosa*	Gonder	gv34	12°11′40N, 037°40′53E	1,827
35	*Guizotia villosa*	Gonder	gv35	12°13′43N, 037°37′44E	1,894
36	*Guizotia scabra* ssp. *scabra*	Jimma	gsc36	07°59′55N, 037°26′22E	1,929
37	*Guizotia scabra* ssp. *scabra*	Jimma	gsc37	08°09′47N, 037°32′09E	1,711
38	*Guizotia scabra* ssp. *scabra*	Jimma	gsc38	07°40′43N, 036°53′59E	1,819
39	*Guizotia scabra* ssp. *scabra*	IllubaborI	gsc39	07°56′19N, 036°30′44E	1,751
40	*Guizotia scabra* ssp. *scabra*	Illubabor	gsc40	08°00′44N, 036°28′26	1,706
41	*Guizotia scabra* ssp. *scabra*	Wellega	gsc41	09°05′59N, 036°58′26E	1,849
42	*Guizotia scabra* ssp. *scabra*	Wellega	gsc42	09°04′10N, 036°54′51	1,767
43	*Guizotia arborescens*	Kaffa	gar43	Omo Nada, on the hill 27 km from the town	2,325–2,420
44	*Guizotia zavattarii*	Sidamo	gz44	Yabelo, 3–7 km from the town on the road to Konso	2,000–2,050
45	*Guizotia zavattarii*	Sidamo	gz45	3 km from Mega to Moyale	1,700–1,750

Table 2 ISSR primers used in the analysis and the number of bands obtained along with the mean Shannon-Weaver diversity index (H′)

Primer code	Sequence	Bands generated		H′ (mean ± se)	PIC (mean ± se)
		Tot	poly		
UBC 834	$(AG)_8$ YT	31	29	0.7300 ± 0.0475	0.3373 ± 0.0094
UBC 841	$(GA)_8$ YC	33	32	0.7258 ± 0.0483	0.3447 ± 0.0096
UBC 866	$(CTC)_5$	27	26	0.6693 ± 0.0480	0.3298 ± 0.0112
UBC 878	$(GGAT)_4$	25	23	0.5997 ± 0.0577	0.2972 ± 0.0126
UBC 888	$[BDB(CA]_7$	29	28	0.7809 ± 0.0486	0.3971 ± 0.0097

Y pyrimidine (C or T), *B* non A (C, G or T), *D* non C (A, G or T), *tot* total number of bands, *poly* number of polymorphic bands

(Gst), were analyzed with DISPAN software (1993), from the single population gene frequencies computed by POPGENE software 1.31. The mean Shannon-Weaver diversity indices were calculated following the procedure of Assefa et al. (2002).

Results

Polymorphism and genetic diversity

Five ISSR primers amplified a total of 145 scorable bands (Table 2). The highest number of bands was scored for *G. scabra* ssp. *schimperi* of which 88% were polymorphic, followed by *G. scabra* ssp. *scabra* with 83.5% polymorphic bands. The least polymorphism (68.2%) was observed in *G. arborescens*. The coefficient of genetic differentiation for the *G. scabra* ssp. *schimperi*, *G. scabra* ssp. *scabra*, *G. villosa* and *G. zavattarii* populations were 0.3610, 0.2552, 0.2551 and 0.0985, respectively, while the total genetic diversity was 0.2401, 0.1868, 0.2117 and 0.1791 for *G. scabra* ssp. *schimperi*, *G. villosa*, *G. scabra* ssp. *scabra* and *G. zavattarii*, respectively. The within population genetic diversity was observed to be higher than the among population genetic diversity for *G. scabra* ssp. *scabra*, *G. scabra* ssp. *schimperi*, *G. villosa*, and *G. zavattarii* (Table 3). The most diverse among its populations was *G. scabra* ssp. *shimperi* with a Dst (among population genetic diversity) of 0.0867 followed by *G. scabra* ssp. *scabra*, *G. villosa* and *G. zavattarii* with Dst of 0.0540, 0.0476 and 0.0177 respectively. The mean Shannon-Weaver diversity indices for the taxa ranged from 0.5791 (*G. arborescens*) to 0.7373 (*G. scabra* ssp. *schimperi*) (Table 3), while the average heterozygosity ranged from 0.1867 (*G. villosa*) to 0.2410 (*G. scabra* ssp. *schimperi*).

Table 3 Genetic diversity and the Shannon-Weaver diversity index for the *Guizotia* species investigated

Taxa	Gst	Ht	Hs	Dst	H′
G. scabra ssp. *schimperi*	0.3610	0.2401	0.1534	0.0867	0.7373
G. villosa	0.2551	0.1867	0.1391	0.0476	0.6619
G. scabra ssp. *scabra*	0.2552	0.2117	0.1576	0.0541	0.7313
G. zavattarii	0.0985	0.1791	0.1615	0.0176	0.6564

Genetic distance and gene flow

The standard genetic distance between the *G. scabra* ssp. *schimperi* populations was found to be least between populations from adjacent areas and greatest between those distant from each other (data not shown). Thus, the standard genetic distance for the *G. scabra* ssp. *schimperi* populations ranged from 0.0017 between populations 1 and 2 (gsh1 & gsh2) both from Hararghe region to 0.1754 between population 18 and population 27 (gsh18 and gsh27) from Gojam and Sidamo, respectively. Likewise the standard genetic distance for the *G. villosa* populations ranged from 0.0125 (between gv 31 and gv 32) both from Gojam to 0.0866 (between gv 28 and gv 34) from Gojam and Gonder, respectively, while for *G. scabra* ssp. *scabra* it ranged from 0.097 (between gsc 36 and gsc37) to 0.1076 (between gsc 36 and gsc 42). The standard genetic distance between the two populations of *G. zavattarii* was 0.0214. The Nei's (1972) genetic distance also followed a similar trend for all the individuals of the species (data not shown).

The standard genetic distance among the five taxa ranged from 0.1188 (between *G. scabra* ssp. *schimperi* and *G. villosa*) to 0.2740 (between *G. scabra* ssp. *schimperi* and *G. zavattarii*) (Table 4). The amount of gene flow among *G. scabra* ssp. *schimperi*, *G. villosa*, *G. scabra* ssp. *scabra* and *G. zavattarii* populations, estimated as $N_m = 0.5(1 - Gst)/Gst$ was found to be 0.8849, 1.5473, 1.6782 and 4.576, respectively.

The UPGMA dendrogram (Fig. 1) based on pairwise comparison of genetic distances produced five separate clusters, one for each of the five taxa.

Table 4 Standard genetic distances of the *Guizotia* species used for the study

	G. scabra ssp. *schimperi*	*G. villosa*	*G. scabra* ssp. *scabra*	*G. zavattarii*
G. villosa	0.1188			
G. scabra ssp. *scabra*	0.1899	0.2729		
G. zavattarii	0.2535	0.2602	0.1819	
G. arborescens	0.2740	0.2555	0.2553	0.1716

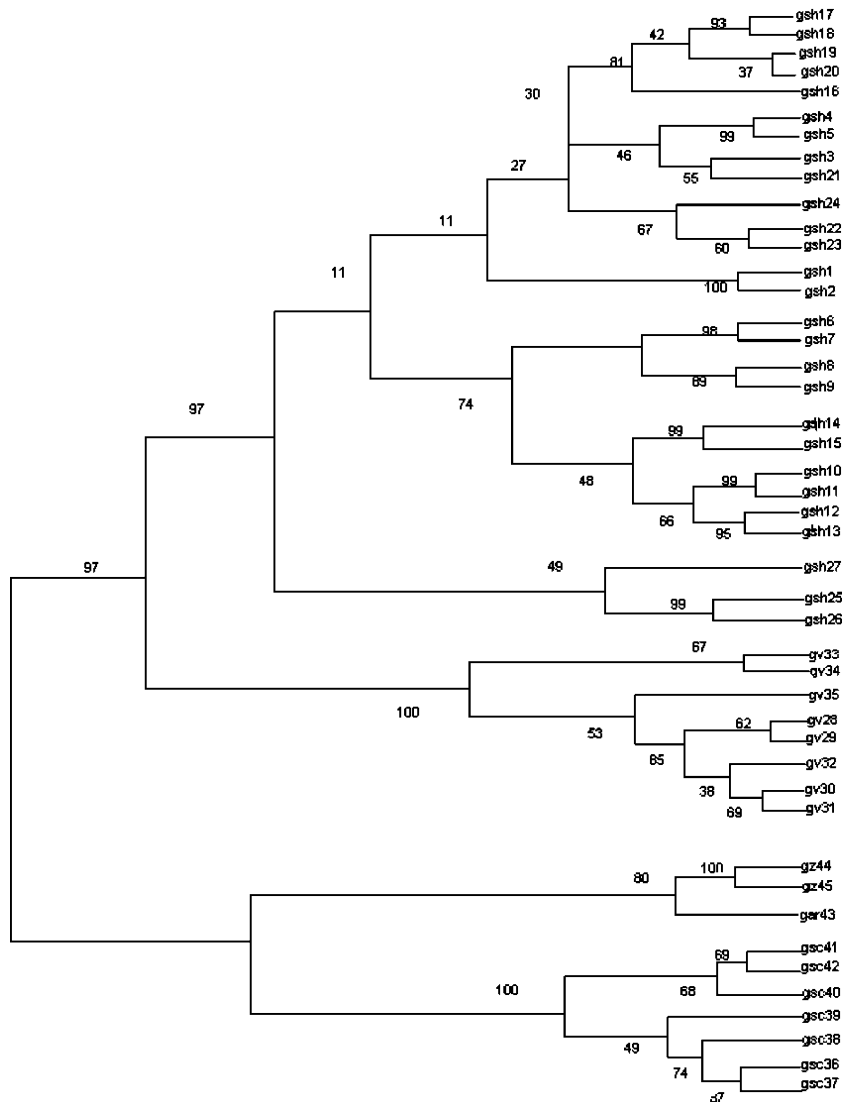

Fig. 1 Clustering pattern of 45 populations of wild *Guizotia* species generated by UPGMA cluster analysis. gsh – *G. scabra* ssp. *schimperi*, gsc – *G. scabra* ssp. *scabra*, gv – *G. villosa*, gar – *G. arborescens*, gz – *G. zavattarii*

The extent of genetic relatedness between populations of a species is revealed as populations from nearby areas are placed nearer to each other forming smaller clusters in the major clusters containing the specific taxa. The genetic affinities and relatedness of the five taxa under consideration can also be observed from the UPGMA clustering based on the standard genetic distances between the five taxa (Fig. 2). Thus two clusters, one consisting of *G. scabra* ssp. *schimperi* and *G.villosa* and the other consisting of *G. arborescens*, *G. zavattarii* and *G. scabra* ssp. *scabra* can be observed.

Discussion

The high polymorphism observed in *G. scabra* ssp. *schimperi* is attributed to the countrywide distribution of this species and the wide distance separating the collection sites of its populations included in the study covering most of the regions in the country (Table 1). *G. scabra* ssp. *scabra* is the second most polymorphic. In fact it is also the second most widely distributed in Ethiopia among the five *Guizotia* taxa studied. The least polymorphic of the five taxa studied was *G. arborescens* followed by *G. zavattarii*. This can likewise be explained by the lesser extent of distribution of these two species in the country. Overall *G. scabra* ssp. *schimperi* is more variable with respect to percent polymorphic loci, total gene diversity, the mean Shannon-Weaver Diversity Index and the range of standard genetic distance among its population.

It may be interesting to consider the amount of gene flow among the populations of these taxa in the light of the genetic distances among the populations of the respective taxa. Overall, the genetic distance

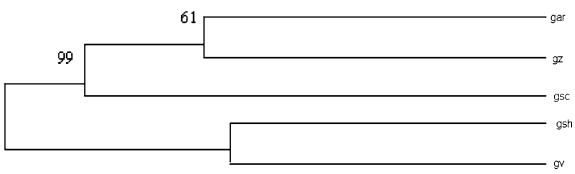

Fig. 2 Unweighted pair group method using the arithmetic average clustering of the five Guizotia taxa based on the standard genetic distance gsh— *G. scabra* ssp. *schimperi*, gsc — *G. scabra* ssp. *scabra*, gv— *G. villosa*, gar— *G. Arborescens*, gz— *G. zavattarii*

was found to be greater and the amount of gene flow less among the populations of *G. scabra* ssp. *schimperi* populations than among the populations of *G. scabra* ssp. *scabra*, *G. villosa* and *G. zavattarii* populations. In fact the higher range of variation in the standard genetic distance among the *G. scabra ssp. schimperi* populations is the result of the greater distance maintained by population 27 (gsh 27) from the others attaining a distance of 0.1754 with population 18 (gsh 18). The standard genetic distance between gsh 27 and the rest of the *G. scabra* ssp. *schimperi* populations ranged from 0.0488 with population 26 (gsh 26) to 0.1754 with gsh 18. This population is even more distant from all the populations of the other taxa studied. Thus, the highest standard genetic distances between gsh 27 and the other taxa were 0.2677, 0.4081, 0.44574 and 0.4044 between *G. villosa*, *G. scabra* ssp. *scabra*, *G. arborescens* and *G. zavattarii* respectively, revealing its affinity with the *G. scabra* ssp. *schimperi* populations and its distance from the other *Guizotia* taxa.

The standard genetic distance between the *Guizotia* taxa studied based on the grouped gene frequency reveal that the distance is least between *G. scabra* ssp. *schimperi* and *G. villosa* (0.1188) and highest between *G. scabra* ssp. *schimperi* and *G. zavattarii* (0.2740). The UPGMA dendrogram based on the standard genetic distances for the five species also elucidate this fact (Fig. 2). On the dendrogram *G. scabra* ssp. *schimperi* and *G. villosa* were clustered together, while *G. zavattarii*, *G. arborescens* and *G. scabra* ssp *scabra* were grouped together.

Our analysis reveals that *G. scabra* ssp. *schimperi* and *G. villosa* share more features in common than with any of the other three species. Thus based on the amount of bands shared, and the standard genetic distance, *G. scabra* ssp. *schimperi* is more closely related to *G. villosa* than to *G. scabra* ssp. *scabra*. Apart from their similarity at the molecular level, these two species share many other characteristics including similar karyotypes (Hiremath and Murthy 1992), herbaceous growth habit, being annual plants and both being common weeds in fields under cultivation, very often occurring together in the same field. It has already been shown by Dagne (1994b) that these species can form interspecific hybrids in areas where they grow together.

Based on this study, the authors recommend the revision of the previous classification that merged the

two taxa as sub species of *G. scabra*. The authors also suggest that *G. scabra* ssp. *schimperi* merits its own specific status as the result of the present investigation and earlier reports (Murthy et al. 1993; Getinet and Sharma 1996). However, the classification of *G. scabra* ssp. *schimperi* as a subspecies of *G. abyssinica*, as suggested by some authors (Murthy et al. 1995), may not be a solution because the International Rules of Botanical Nomenclature would not support the merger of a wild species with a cultivated one.

Acknowledgements The authors would like to extend their gratitude to the Swedish University of Agricultural Sciences and the Alemaya University because of whose cooperation this study was made possible. We are very grateful to Dr Kifle Dagne of the Addis Abeba University for his encouragement and providing important materials. Special thanks also go to Dr Kebebew Assefa, Mr Faris Hailu and Mr Mulatu Geleta who were always available for help and advice.

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