Analysis of genetic diversity of *Guizotia abyssinica* from Ethiopia using inter simple sequence repeat markers

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Within and among population genetic diversity of 37 *Guizotia abyssinica* populations from Ethiopia were analyzed using inter simple sequence repeats (ISSRs). Five primers amplified a total of 118 genomic DNA fragments across a total of 370 individuals of which 106 were polymorphic (89.83%). The average number of polymorphic bands per primer was 21.2. More bands were generated by primer UBC 888 (BDB(CA)$_7$. The total genetic diversity (Ht) and the coefficient of genetic differentiation (Gst) were 0.4115 and 0.0918 respectively, while the within population genetic diversity (Hs) and the among population genetic diversity(Dst) were 0.3738 and 0.03776 respectively suggesting more variability within the populations than among them. The standard genetic distances between the *G. abyssinica* populations of the eight regions ranged from 0.0281 (between Wollo and Gojam) to 0.1148 (between Jimma and Hararghe). Generally, the standard genetic distances are smaller between populations of neighboring regions and highest between those of Jimma and the other regions, ranging from 0.0696 (between Jimma and Shewa) to 0.1148 (between Jimma and Hararghe). The ISSR based UPGMA clustering using the standardized genetic distances matrix also placed populations from neighboring regions closer than those from farther apart areas, while the UPGMA clustering by regions based on the standard genetic distances produced three clusters following the proximity and the contiguity of the regions. The mean Shannon Weaver diversity indices for the populations of the eight regions ranged from 0.8197 (Jimma) to 0.9176 (Hararghe), with a mean of 0.8841 for the whole material.

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Guizotia abyssinica (L.f) Cass. (niger in English) 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 major growing areas of niger as edible oil seed are Ethiopia and the Indian sub continent (MURTHY et al. 1993), though it is reported to be grown as a minor oil seed crop also in Sudan, Tanzania, Uganda and Malawi (RILEY and BELAYNEH 1989). In Ethiopia, it is cultivated mainly in Gojam, Shewa, Wellega and Gonder regions and to a lesser extent in Jimma, Wollo, Arsi and Hararghe regions (GETINET and SHARMA 1996).

It is asserted that *Guizotia abyssinica* has its center of diversity in Ethiopia (BAAGOE 1974; MURTHY et al. 1993), and believed to have its center of origin also in Ethiopia (HIREMATH and MURTHY 1988). MURTHY et al. (1993) showed the similarity and the homologous nature of the genomes of *G. abyssinica* and *G. scabra* ssp *schimperi* based on cytological studies and asserted that *G. abyssinica* might have evolved from *G. scabra* ssp *schimperi* in northern Ethiopia through selection and cultivation of large achene mutants. HIREMATH and MURTHY (1988) also suggested the evolution of *G abyssinica* from *G scabra* ssp *schimperi* in Ethiopia based on phytogeographic, morphological and cytogenetic evidences.

Niger is the only cultivated species of the genus *Guizotia*. It is a diploid plant with a chromosome number of $2n = 2x = 30$ (DAGNE 1994). The plant height varies depending on the environmental conditions of the growing area. It grows to an average height of 1.2 m, though a height of 2.1 m is also observed depending on the growing condition. Niger is an annual plant with a hollow stem. It is highly cross pollinated and self incompatible (HIREMATH and MURTHY 1986; ADDA et al. 1994). Thus growers may be recommended to have bee hives close to the growing area of the crop to facilitate cross pollination by bees. It is a low yielding crop with low fertility needs. The low yield may be attributed to the self incompatibility nature of the crop and the low input condition under which it is generally grown.

According to GETINET and SHARMA (1996), the niger populations in Ethiopia fall into three maturity groups referred to as 'Bunigne' niger, 'Mesno' niger and 'Abat' niger. Bunigne is the early maturing type with a shorter growing period of about four months (July to October), while Abat niger takes about seven months to mature (June to December). Mesno niger is late maturing (September to February) but frost

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resistant unlike the other two (Getinet and Sharma 1996).

Generally, there is not much work done on niger. An in-depth treatment of its taxonomy and distribution was done by Baagoe (1974). Cytological studies were done by several workers (Hiremath and Murthy 1986; Dagne and Heneen 1992; Hiremath and Murthy 1992; Dagne et al. 2000; Dagne 2001). However, to date there is no report of using ISSR markers to study the genetic diversity of niger. ISSR markers, like any other PCR-based marker, are rapid and require only small amount of the template DNA. Each marker system has its own advantages and disadvantages. RAPD's lack reproducibility (Virk et al. 2000; Bornet and Branchard 2001), and AFLP has high operational cost (Prevost and Wilkinson 1999). Microsatelites, though highly polymorphous, require prior knowledge of the genomic sequence to develop specific primers and are thus limited to economically important plants (Bornet and Branchard 2001). Inter simple sequence repeat markers with low cost and low labor requirement but with high reliability have been developed since 1994 (Zietkiewicz et al. 1994). ISSR amplification does not require genome sequence information but produce highly polymorphic patterns (Zietkiewicz et al. 1994; Nagaoka and Ogihara 1997; Prevost and Wilkinson 1999). They seem to have the reproducibility of SSR's and the usefulness of RAPD's (Bornet and Branchard 2001), and thus combine the advantages of SSR and AFLP and the utility of RAPD. ISSR markers have been used to determine the genetic diversity of *Eragrostis tef* (Assefa et al. 2003), Cicer (Sudupak 2004) and wild rice (Qian et al. 2001). It was also applied to the study of genetic relationships and phylogenetic analysis of various crop plants (Joshi et al. 2000; Martin and Yelemo 2000; Iruela et al. 2002). ISSR is a technique that is gaining wide acceptance in the area of plant improvement. Its utility in crop improvement by plant breeding makes use of the fact that certain DNA markers are closely linked to important agronomic traits (Reddy et al. 2002). Thus it has been widely used to identify markers associated with different qualities in crop plants such as disease tolerance and seed size (Ammiraju et al. 2001).

The present work is an attempt to study the genetic diversity among the niger populations grown in different regions of Ethiopia using ISSR markers in an effort to provide some information for future research that might be aimed at improving some of the agronomic traits of niger for efficient utilization and conservation.

MATERIAL AND METHODS

The plant material and DNA extraction

The plant material used in the study include 10 individuals of *Guizotia abyssinica* (L.f) Cass. from each of the 37 populations (accessions) collected from eight niger growing regions in Ethiopia (Table 1, Fig. 1). As niger is grown only in localized areas and in much smaller scales in some of the regions, only 3, 4 and 5 populations were collected from Jimma, Arsi and Hararghe respectively. Twenty populations were collected from each of the main niger growing regions (Gojam, Gonder, Wellega, Shewa and Wollo. However, only five populations that were physically located farther apart from each of these regions were selected for analysis. This was meant to make the sample size of all the regions match for a better comparison of the

Table 1. *Regions and site coordinates of the G. abyssinica populations studied.*

Region	Population code	Site coordinates	Altitude(m)
Shewa	Asfachew	9°53′N, 39°5′E	1009
	Kobo	8°42′N, 38°15′E	2155
	Soyama	8°25′N, 37°53′E	1900
	Yaya	9°42′N, 38°49′E,	2669
	Worku	9°45′N, 38°46′E	2674
Jimma	Tiyyo	7°52′N, 37°16′E	1760
	Dacha	7°53′N, 37°17′E	1748
	Ayno	7°52′N, 37°17′E	1725
Wellega	Kane	9°3′N, 36°29′E	2176
	Qawissa	8°58′N, 36°29′E	2240
	Damasa	8°59′N, 36°30′E	2260
	Ale	8°57′N, 36°29′E	2252
	Jirata	9°2′N, 36°29′E	2142
Arsi	Gobessa	7°36′N, 39°31′E	2374
	Jelko	7°26′N, 39°32′E	2352
	Tareta	7°35′N, 39°33′E	2336
	Shirka	7°36′N, 39°34′E	2334
Wollo	Koladi	10°52′N, 39°49′E	2374
	Sedeko	10°29′N, 39°56′E	1551
	Kombolcha	10°59′N, 39°46′E	1767
	Gerado	11°45′N, 39°37′E	1913
	Libso	11°34′N, 39°40′E	1662
Gonder	Tamo	12°6′N, 39°46′E	1894
	Anguabo	11°56′N, 37°48′E	1947
	Zuria	12°22′N, 37°33E	1942
	T/H	12°32′N, 37°26′E	1895
	Azezo	12°29′N, 37°27′E	1898
Gojam	Kotkotuma	11°27′N, 37°14′E	2021
	Awabel	10°13′N, 38°8′E	2466
	Yabesh	10°37′N, 37°31′E	2097
	Nifasam	10°17′N, 37°48′E	2463
	Rufael	11°30′N, 37°24′E	1793
Hararghe	Makanisa	8°53′N, 40°43′E	1714
	Makana	8°54′N, 40°46′E	1702
	Kara	8°52′N, 40°40′E	1752
	Haro	8°52′N, 40°37′E	1747
	Bareda	8°51′N, 40°38′E	1746

Fig. 1. Geographycal map of Ethiopia indicating the regions of origin of the niger samples used in the study.

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

PCR amplification and electrophoresis

PCR was performed by means of five ISSR primers that were selected out of fifteen 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. Each primer was tested for reproducibility for different PCR products of DNA samples of the same population and separate runs on polyacrylamide gels and the ones producing consistent DNA fragments across the different samples and PCR runs were selected.

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), 2m M $MgCl_2$, 0.2 mM of the dNTP's (dATP, dCTP, dGTP, dTTP), 2% formamide, 0.2 µM primer, 0.05 U μl^{-1} 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.

Table 2. *ISSR primers used in the analysis and the number of bands obtained along with the mean Shannon Weaver diversity index (H′) and the polymorphism information content (PIC) for each.*

Primer code	Primer sequence	Bands generated		H′ (mean ± se)	PIC (mean ± se)
		Tot	Poly		
UBC 834	$(AG)_8$ YT	26	23	0.950 ± 0.012	0.466 ± 0.008
UBC 841	$(GA)_8$ YC	25	25	0.971 ± 0.008	0.480 ± 0.006
UBC 866	$(CTC)_5$	19	18	0.969 ± 0.008	0.479 ± 0.006
UBC 878	$(GGAT)_4$	20	17	0.938 ± 0.017	0.459 ± 0.011
UBC 888	$(BDB(CA)_7$	28	23	0.964 ± 0.007	0.476 ± 0.005

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

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 the NEI (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 distance matrices among the eight regions' populations and the resulting distance matrices used to construct an unweighted pair group method using the arithmetic average (UPGMA) phenogram for the populations in the eight regions. The NEI (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 (Gst), were analyzed with the same software package from the single population gene frequencies computed by POPGENE software 1.31 (YEH et al. 1999). The mean Shannon weaver diversity indices were calculated following the procedure of ASSEFA et al. (2002).

RESULTS

ISSR analysis using five primers produced a total of 118 scorable fragments of which 106 (89.83%) were polymorphic. Of the five primers used in the experiment, UBC 888 ($BDB(CA)_7$ produced more bands while UBC 841 $(GA)_8YC$ produced more polymorphic bands. The size of the bands produced by these primers ranged from about 50 bp to about 1500 bp. The coefficient of gene differentiation (Gst) for all loci was 0.0918, while the estimate of the total genetic diversity (Ht) was found to be 0.4115. The among population genetic diversity (Dst) was 0.0378, and the within population genetic diversity (Hs) was 0.3738. The within population genetic diversity is highest for the populations from Wollo region, while the lowest among population genetic diversity was observed among the Gojam populations (Table 3). The amount of gene flow among these populations, estimated as $N_m = 0.5(1 - Gst)/Gst$ was found to be 2.3716.

The measure of genetic distance for all populations used in the study (NEI 1972) was lowest (0.0503) between the populations kombolcha and Gerado, both from Wollo region, and highest (0.3261) between populations Tiyyo and Koladi from Jimma and Wollo regions respectively. The standard genetic distances for the regions' populations ranged from 0.0281 (between Wollo and Gojam) to 0.1148 (between Jimma and Hararghe) (Table 4). The average heterozygosity ranged from 0.3632 (Wellega) to 0.4187 (Shewa), while the estimates of the mean Shannon Weaver diversity index for the data was 0.8841. When analysed by region, the mean Shannon weaver diversity indices ranged from 0.8197 for Jimma to 0.9176 for Hararghe (Table 4)

Table 3. *Genetic diversity and the Shannon weaver diversity index for the niger populations of each region.*

Region	Gst	Ht	Hs	Dst	H′
Wolo	0.1143	0.3735	0.3308	0.0427	0.9102
Gonder	0.1806	0.3583	0.2936	0.0647	0.8827
Gojam	0.2090	0.3375	0.2968	0.0407	0.9062
Hararghe	0.1743	0.3810	0.3146	0.0664	0.9176
Shewa	0.1915	0.4088	0.3305	0.0783	0.9080
Jimma	0.1738	0.3703	0.3059	0.0644	0.8197
Wollega	0.1609	0.3559	0.2987	0.0573	0.8627
Arsi	0.1896	0.3688	0.2989	0.699	0.8656

A UPGMA dendrogram based on pairwise comparison of genetic distances identified three major clusters (Fig. 2). Populations from the northern and eastern regions (Wollo, Gonder, Gojam and Hararghe) were grouped together in one cluster, while the Shewa (central), Wellega (western) and Arsi (southern) populations formed a separate cluster. Two populations from south west Shewa and a population from Jimma formed a small cluster removed from the major two. The clustering pattern for the region's populations based on the standard genetic distances also reveal the formation of three clusters with Gojam, Gonder, Wollo and Hararghe forming one cluster, and Wellega, Shewa and Arsi the other, while Jimma forms a separate cluster of its own (Fig. 3).

DISCUSSION

ISSR analysis is a powerful tool for assessing the genetic diversity in *G. abyssinica*. This fact is evidenced by the detection of high level of polymorphism in this species using ISSR primers. In the present study the three genetic diversity indices; percent polymorphic loci, average heterozygosity and the Shannon Weaver diversity indices proved that the genetic diversity in the niger populations of Ethiopia is indeed high. Of the total gene diversity (Ht) which was 0.4115, the within population genetic diversity accounts for about 90.8%, while the among population genetic diversity takes only a small fraction of the total

Table 4. *Standard genetic distances of the G. abyssinica populations analysed by region.*

Region	Wollo	Gonder	Gojam	Hararghe	Shewa	Jimma	Wellega	Arsi
Wollo	0							
Gonder	0.0308	0						
Gojam	0.0281	0.0289	0					
Hararghe	0.0394	0.0388	0.0336	0				
Shewa	0.0428	0.0589	0.0440	0.0499	0			
Jimma	0.1007	0.1018	0.1005	0.1148	0.0696	0		
Wellega	0.0669	0.0589	0.0682	0.0716	0.0522	0.0505	0	
Arsi	0.0491	0.0508	0.0503	0.0468	0.0391	0.0951	0.0355	0

Ht. This is characteristic of cross pollinating species. In fact niger is highly cross pollinating and self incompatible (GETINET and SHARMA 1996, HIREMATH and MURTHY 1986). The amount of gene flow among these populations was estimated as $N_m = 2.3716$. As N_m is indicative of the number of migrants (JIAN et al. 2004), it suggests that the average number of migrants per generation (N_m) between the niger populations of Ethiopia included in the present study is 2.3716. About similar figure is obtained by using the formula of SLATKIN and BARTON (1989) for the amount of gene flow. Gene flow is found to be highest for the populations from Wollo region ($N_m = 3.8439$) and lowest for the populations from Gojam followed by those from Shewa with N_m of 1.9461 and 2.0488 respectively (data not shown). This becomes very interesting when one considers the geographic distance between the collection sites from these regions with lower N_m. The samples from Gojam and Shewa were very much removed from each other within their respective regions as compared to those with relatively higher gene flow values (Table 1)

While the general trend in the UPGMA clustering is that of grouping populations by region of origin and proximity of geographic location of the collection sites, not all populations, however, belonging to the same region were grouped together in the same cluster. Thus the population 'Awabel' from Gojam is included in the cluster containing Shewa, Wellega and Arsi, while two populations from Arsi (Shirka and Tareta)

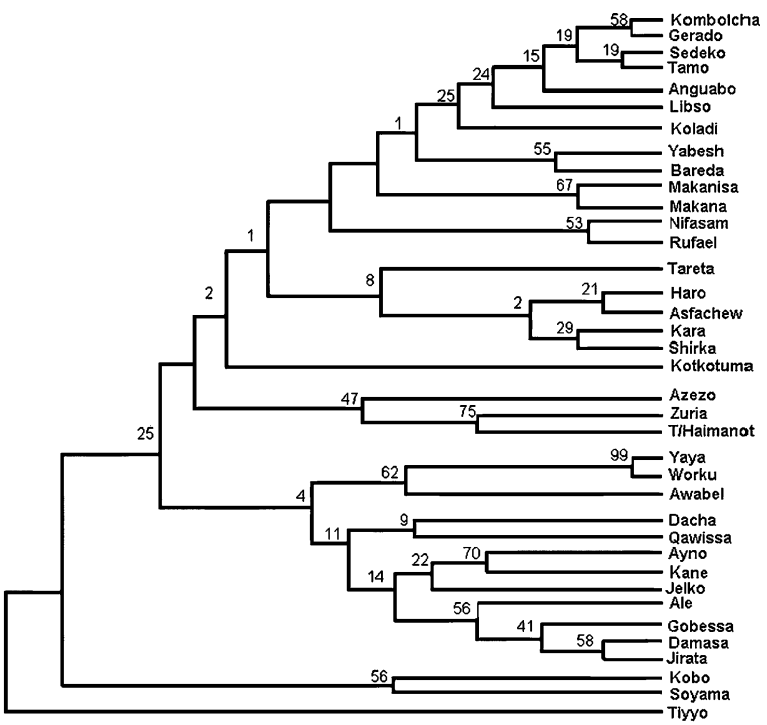

Fig. 2. Dendrogram illustrating the clustering pattern of 37 populations of *Guizotia abyssinica* generated by UPGMA cluster analysis of 106 ISSR markers.

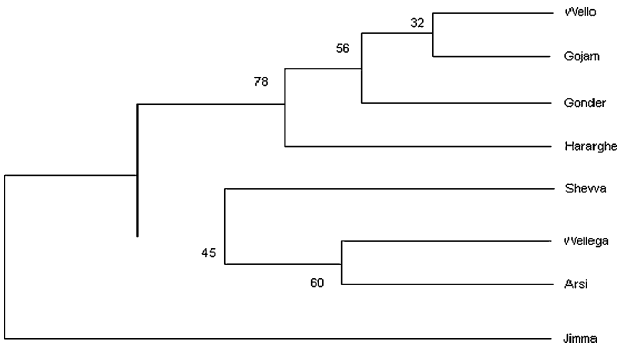

Fig. 3. Clustering by region based on the standard genetic distances for the niger populations from Ethiopia

were included in the cluster containing the populations of the northern and eastern regions. This may be due to either or both of the following reasons:

- the continuity of the niger growing regions of Ethiopia which makes possible the transfer of seed materials from one region to the other. In fact, samples collected from the border of a region are closer by proximity of geographic location to the adjoining region than to the collection sites of the same region. For example, the population Awabel collected from southern Gojam is grouped with Yaya and Worku collected from northern Shewa bordering Gojam. It is thought that though the Abay valley separates northern Shewa from southern Gojam material transfer between these two areas is highly likely.
- The existence of three strains of niger that are differentiated on the basis of the duration to maturity identified as 'bunigne', 'abat' and 'mesno'.

In fact, the field observation of these same samples reveals the existence of niger strains that are widely different in the duration to flowering and maturity. According to the field observation all samples from Wollo and Hararghe are of the early maturing type, and only one sample from Shewa (Asfachew) and two samples from Gonder (Tamo and Anguabo) are of the early maturing types, while the samples from Gojam, Wellega, Jimma Arsi and Shewa are of the Abat (late maturing) types. The clustering in the dendrogram (Fig. 2) seems, to a certain extent to follow this trend while the general trend is that of clustering the populations by their region of origin.

Thus it appears that the 'Bunigne' populations of a region are grouped differently from the 'Abat' populations of the same region in the dendrogram. However, the confirmation of this assertion needs further investigation. Most of the niger grown in Wollo and Gonder is reported to be of the 'Bunigne' type, while those in Wellega and Gojam of the 'Abat' type (Getinet and Sharma 1996).

The standard genetic distance between the Jimma populations and those from the rest of the regions are large indicating a sort of genetic isolation of the Jimma population from those of other regions. Indeed in the dendrogram clustering (Fig. 3), the Jimma populations formed a separate cluster. Looking at the geographic location of Jimma region would explain this fact. Jimma is situated between Wellega and Shewa, but the niger growing areas of Jimma are not contiguous with the niger growing areas of Wellega and Shewa as expanses of two great arid valleys separate it from these regions. On the north west the Diddessa valley (Diddessa desert as it is commonly called) separates Jimma from Wellega and on the north east side the Ghibe valley (also called the Ghibe desert) separates Jimma from Shewa. Thus, it is no wonder that the niger populations from Jimma are removed from the others on the basis of the dendrogram grouping maintaining higher genetic distances with all the populations from the other regions.

Overall our findings demonstrated that there is variation within the niger populations of Ethiopia and that ISSR markers would be useful for the assessment of genetic diversity and phylogenetic relationships of this species.

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