SINET: Ethiop. J. Sci., 27(2):143–152, 2004
 ISSN: 0379–2897

ISOENZYME ANALYSIS OF FIVE ENDEMIC AND ONE WIDESPREAD *KNIPHOFIA* SPECIES (ASPHODELACEAE) OF ETHIOPIA

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ABSTRACT: There are seven species of genus *Kniphofia* in Ethiopian flora. *K. foliosa, K. hildebrandtii, K. insignis, K. isoetifolia,* and *K. schimperi* are endemic. *Kniphofia pumila* and *K. thomsonii* are widespread from West Africa to Eastern and Central Africa. In this study the genetic diversity and the relationships between five endemic and one widespread *Kniphofia* species in Ethiopia was conducted based on isoenzymes systems. The electrophoretic analyses of four enzymes systems in the species from ten experimental sites in ten locations revealed seven putative loci, of which PGM-1, PGM-2, AAT-2 and GPI-2 were polymorphic. The species have displayed higher genetic diversity parameters than other endemic plants. The over all mean inbreeding coefficient (*F*) was positive indicating slight deficiency in the number of heterozygotes. The total genetic diversity varies from 0.5 in *K. schimperi* to 1.11 in *K. isoetifolia* indicating very low diversification between the populations with in a species. At the generic level H_T ranges from 0.034 to 0.470 with a mean of 0.247. The genetic variation among the species (G_{ST}) is 6.6%. Over all mean of genetic distance (0.011) and genetic similarity (0.938) indicated high similarity among the populations. Despite morphological variation particularly in floral morphology and inflorescence architecture, the over all mean of unbiased genetic identity of the species (0.989) is much higher than described for congeneric species (I = 0.64). The results, therefore, indicate that the Ethiopian *Kniphofia* species share a fairly recent common ancestor, but have differentiated in floral and inflorescence character through rapid evolution. This hypothesis needs, however, further testing.

Key words/phrases: Endemism, genetic diversity, isoenzyme, *kniphofia*, inbreeding coefficient

INTRODUCTION

The genus *Kniphofia* Moench belongs to the family Asphodelaceae. Codd (1968) indicated that the genus *Kniphofia* is represented by 70 species. Forty-five species occur in South Africa. One species occurs in Arab Republic of Yemen, 2 in Malagasy Republic, and 23 in Tropical Africa. Sebsebe Demissew and Nordal (1997) recognized seven species of genus *Kniphofia* in the Ethiopian flora. They are *Kniphofia foliosa* Hochst., *K. hildebrandtii* Cufod., *K. insignis* Rendle, *K. isoetifolia* Hochst., *K. pumila* (AIT.) Kunth, *K. schimperi* Baker and *K. thomsonii* Baker. *K. foliosa, K. hildebrandtii, K. insignis, K. isoetifolia,* and *K. schimperi* are endemic to Ethiopia. *Kniphofia pumila* and *K. thomsonii* is widespread (widely distributed) from West Africa to Eastern and Central Africa (Marais, 1973).

The genus *Kniphofia* is useful in the field of Horticulture and is grown in home and botanical gardens, and naturally occurring species of *Kniphofia* are important as honeybee-plants for pollen source and nectar (Fichtl and Admassu Adi, 1994). The secondary metabolites from genus *Kniphofia* such as knipholone and related natural phenylanthraquinones, are considered to be a new group of potential antimalarials and anthraquinone aloe-emodin is known to exhibit antileukemic properties (Ermias Dagne and Steglich, 1984; Esayas Berhanu *et al*., 1986; Bringmann *et al*., 1999). Also, the roots of *Kniphofia foliosa* are used in traditional Ethiopian medicine for treatment of abdominal cramps.

These are distributed between 6° 00' N to 14° 00' N latitude and 33° 00' E to 41° 46' E longitude that falls within the mountainous area of the country with disjunctive distribution. *K. foliosa*, *K. isoetifolia* and *K. schimperi*, unlike *K. insignis* and *K. hildebrandtii*, are not geographically restricted to the central highlands. Their habitat varies from montane grassland (characterised by *Olea europaea* subsp. *cuspidata, Juniperus procera, Celtis africana,*

Euphorbia ampliphylla, Carissa edulis, Rosa abyssinica, Mimusops kummel and *Ekebergia capensis*), to sub-alpine *Erica arborea* zone (characterized by *Erica arborea, Lobelia cynchopetalum* and species of grasses mainly of *Festuca, Poa* and *Agrostis*).

The objective is to study the genetic variation and relation among and within species and populations. Previously no such studies have been conducted although the basic taxonomy has been studied as part of the Ethiopian Flora Project. The results may assist future conservation plan of the species.

MATERIALS AND METHODS

Biological material

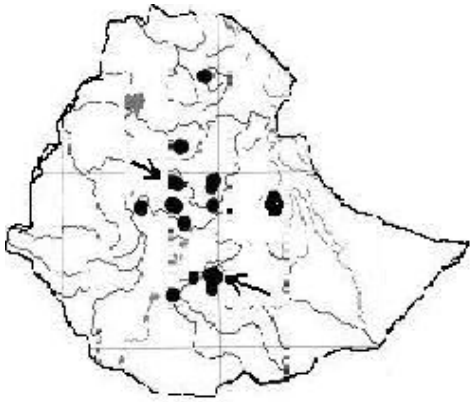
The following species were included in the isoenzyme analysis: *Kniphofia foliosa*, *K. hildebrandtii, K. insignis, K. isoetifolia, K. schimperi and K. pumila*. The plants were grown in a green-house at the University of Oslo from seeds randomly collected from the experimental plots in ten localities in Ethiopia (Table 1 and Fig. 1). The seeds from the individual genet and as well from individual mother plant were collected separately and grown in separate pots. From each population 30 to 90 individuals were analysed. The voucher specimens of the analyzed *Kniphofia* species are kept in National Herbarium of the Addis Ababa University.

Electrophoretic analysis

The isoenzymes analysis procedure was modified from that of Wendel and Weeden (1990) that wicks are not removed from the gels, but resolution is generally of equivalent quality. The starch used for the preparation of the gel was Sigma S-4501. The buffer systems, electrode and gel buffers, were categorized as D-system and AB-systems. The D-system was with modification of Stuber *et al*. (1977) that the pH of the electrode and gel buffers is 6.5. The gel buffer is prepared by adding 125 ml of electrode buffer to 375 distilled water (1:3). AB-system was with modification of Ashton and Braden (1961) that the gel buffer is prepared by diluting 50 ml of electrode buffer (11.87 g/l Boric acid and 1.6 g/l Lithium Hydroxide) in 450 ml of Tris-citrate (6.05g/l Tris-0.31g/l citric acid). The D-system gels were stained for glucose-6-phosphate dehydrogenase (G6PDH 1.1.1.49) and phosphoglucomutase (PGM 5.4.2.2). The AB-system gels were stained for aspartate amino transferase (AAT 2.6.1.1) and glucose-6-phosphate isomerase (GPI 5.3.1.9). Relative mobility (Rf) was calculated based on the migration of a band relative to the front. The alleles at a locus were designated by alphabets. The fastest and the anodal was denoted as 'A' allele and the rest were assigned according to decreasing order of electrophoretic mobility in ascending order of the alphabets.

Table 1. The location of seed collection sites in Ethiopia (voucher number represents the populations**).**

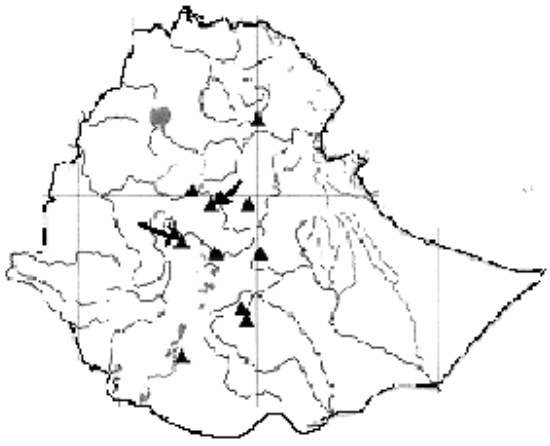
Scientific name	Pop. No.	Region and Locality	Lat. and Long.	Altitude (m.a.s.l.)	Description of habitat
K. foliosa	fol-10	Bale: Dinsho	7º 06' N, 39º 48 E	3500	Montane forest and grassland Dominant plant *Juniperus procera* & *Hagenia abyssinica.*
	fol-13	N. Shewa: Ali Doro	7º 04' N, 39º 47' E	3200	*Erica arborea* zone. Red clay soil with rocky out cropping and surrounded by farmlands.
K. hildebrandtii	hild-4	W. Shewa: Gheddo	9º01' N, 37º 30' E	2450	Over grazed grassland. With newly planted *Eucalyptus* trees. On top of mountain.
K. insignis	ins-8	N. Shewa: Torban Ashie	9º 20' N, 37º 12' E	2800	Meadow waterlogged in the rainy season. Surrounded by farmlands.
	ins-12	E. Shewa: Bull Workie	9º 19' N, 39º 32' E	2750	Meadow waterlogged in the rainy season. Grazing land for sheep enterprise.
K. isoetifolia	iso-1	Bale: Dinsho	7º 08' N, 39º 48' E	3000	Marshy montane grassland.
	iso-2	Arussie: Bekojji	7º 15' N, 38º 53' E	2600	Marshy grassland beside a spring. surrounded by farmlands.
K .pumila	pum-6	Sidamo: Yirba Muda	6º 15' N, 38º 42' E	2700	Red soil. Disturbed part of montane forest.
K. schimperi	sch-5	W. Shewa: Goro Wonchi	9º 46' N, 38º 31' E	3100	*Erica arborea* zone. *Eucalyptus* trees and scattered *Juniperus procera.*
	sch-14	Addis Ababa: Entoto	9º 47' N, 38º 43' E	2820	*Erica arboria* Zone. With scattered *Hagenia abyssinica* and *Juniperus procera.*



K. foliosa: Upper left Ali Doro and lower right Dinsho

K. hildebrandtti: Gheddo

K.insignis: upper left Torban Ashie and right Bull Workie



K. schimperi: lower left: Goro Wonchi and upper right Entoto

Fig. 1 The distribution of endemic *Kniphofia* species in Ethiopia. The arrows indicate the experimental sites. The data to determine the distribution of *Kniphofia* species were collected from the National Herbarium of Addis Ababa University (ETH), Royal Botanical Garden; Kew (K), and of the Natural History Museum of London (BM).

Data analysis

The basic genetic parameters, Hardy-Weinberg test, and *F*-statistics, genetic distance, identity and similarity analysis were calculated, and dendrogram was generated based on a UPGMA analysis (Sokal and Michener, 1958) using BIOSYS-1 release 7.1 (Swofford and Selander, 1989) and POPGENE version 3.1 (Francis and Rong-Cai, 1999) computer programs. BIOSYS-1 release 7.1 and POPGENE version 1.30 are useful in population genetics and related fields. Their major features are for analysis of basic genetic parameters, Hardy-Weinberg test, *F*-statistics, Nei's genetic distances and identities (Nei, 1978), and genetic similarity (Rogers, 1972) between groups or populations. Besides, POPGENE enables to estimate Shannon-Weaver diversity parameters and to generate dendrogram based on a UPGMA analysis (Sokal and Michener, 1958).

RESULTS

Out of the four enzyme systems analysed only PGM, AAT and GPI were polymorphic and interpretable. PGM has three putative loci (Fig. 2). PGM-1 displayed five alleles (A-E). The alleles A, B and C are common and found in all the species. Allele D is less common and only found in *Kniphofia insignis* and *K. schimperi*. Allele E is a rare allele only found in one population of *K. schimperi* ("sch-14", Table 1). PGM-2 displayed three alleles (A-C). Allele B is common in all and fixed in half of the populations. Allele A is found in *K. insignis* and in one population of *K. isoetifolia* ("iso-1"). Allele C is found in *K. isoetifolia* and in one population of *K. foliosa* ("fol-13") and *K. insignis* ("ins-8"). PGM-3 is fixed and monomorphic in all the species (Table 2).

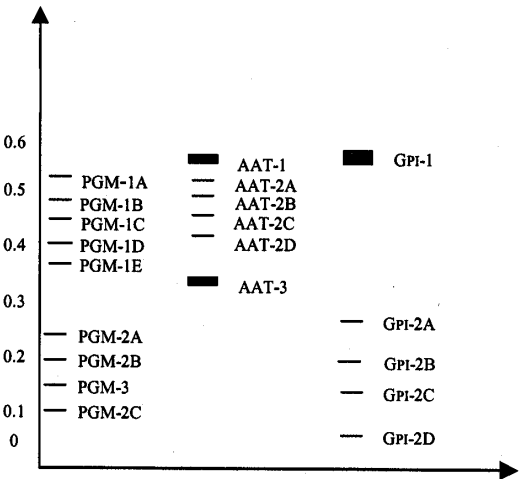


Fig. 2. The relative mobility of the alleles in the variable loci of PGM, AAT and GPI. The shaded bands represent blurred bands.

Table 2. The sample size (N) and mean, standard error (se) of the allele frequencies of polymorphic locus in the populations of genus *Kniphofia* species.

		Population of genus *Kniphofia* species											
Locus	Alleles	fol-10	fol-13	hild-4	ins-8	ins-12	iso-1	iso-2	sch-5	sch-14	pum-6	mean	se
	(N)	39	30	35	31	36	90	36	46	73	51		
PGM-1	A	0.103	0.125	0.071	0.177	0.083	0.150	0.139	0.043	0.048	0.049	0.099	0.047
	B	0.872	0.821	0.757	0.500	0.444	0.733	0.667	0.707	0.603	0.843	0.695	0.143
	C	0.026	0.054	0.171	0.258	0.417	0.117	0.194	0.217	0.281	0.108	0.184	0.117
	D	0.000	0.000	0.000	0.065	0.056	0.000	0.000	0.033	0.062	0.000	0.022	0.029
	E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.001	0.002
PGM-2	A	0.000	0.000	0.000	0.016	0.042	0.011	0.000	0.000	0.000	0.000	0.007	0.004
	B	1.000	0.982	1.000	0.952	0.958	0.956	0.986	1.000	1.000	1.000	0.983	0.006
	C	0.000	0.018	0.000	0.032	0.000	0.033	0.014	0.000	0.000	0.000	0.010	0.004
AAT-2	A	0.000	0.000	0.000	0.000	0.000	0.072	0.083	0.000	0.000	0.000	0.016	0.010
	B	0.949	0.946	1.000	0.968	0.931	0.911	0.889	0.837	0.842	0.980	0.925	0.018
	C	0.000	0.000	0.000	0.000	0.000	0.017	0.028	0.120	0.137	0.000	0.030	0.017
	D	0.051	0.054	0.000	0.032	0.069	0.000	0.000	0.043	0.021	0.020	0.029	0.008
GPI-2	A	0.000	0.000	0.000	0.129	0.125	0.150	0.278	0.000	0.000	0.039	0.072	0.030
	B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.098	0.027	0.000	0.013	0.010
	C	0.962	0.911	0.757	0.677	0.694	0.850	0.722	0.750	0.760	0.961	0.804	0.034
	D	0.038	0.089	0.243	0.194	0.181	0.000	0.000	0.152	0.212	0.000	0.111	0.031

AAT has three putative loci (Fig. 2). The loci identified as AAT-1 and AAT-3 are blurred and not interpretable. The middle locus identified as AAT-2 has four alleles (A-D). Allele B is most common in all species and populations. Allele A is rare and only found in *K. isoetifolia*. Allele C is also rare but found in *K. isoetifolia* and *K. schimperi*. Allele D is found in all the species except in *K. hildebrandtii* and *K. isoetifolia* (Table 2).

GPI has two putative loci (Fig. 2). The anodal locus has bands without sharp distinction. It is assumed to be of chloroplast origin, and was not further interpreted. The cathodal locus GPI-2 has four alleles (A-D). Allele C is the most common in all the species and populations. Allele A is found in *K. insignis, K. isoetifolia and K. pumila*. Allele B is found only in *K. schimperi*. A very faint mark of allele B was observed in *K. pumila* and more rarely in *K. foliosa* when the gel was kept in the stain solution for longer period than usual. These bands were regarded as ghosts and were not scored. Allele D is present in all species except in *K. isoetifolia* and *K. pumila*. A third putative locus was observed towards the cathode from the application point, but was not consistently expressed and accordingly not included in the data or final analysis (Table 2).

G6PDH has two regions identified as G6PDH-1 and G6PDH-2 with Rf values 0.26 and 0.14; respectively. Both were monomorphic in all the samples.

Four loci were polymorphic and they all together displayed 16 allelic bands. At the species level, the percentage of polymorphic loci (P) ranges from 28.6 % in *K. hildebrandtii* to 57.1% in *K. foliosa, K. insignis* and *K. isoetifolia* (Table 3). The lowest mean number of alleles (A) per locus is 1.43 in *K. hildebrandtii* and the highest is 2.14 in *K. insignis* and *K. schimperi. K. foliosa* has the lowest mean number of alleles per polymorphic locus (AP=2.25), and *K. schimperi* has the highest (AP=3.6). The mean effective number of alleles per locus (Ae) range from 1.07 in *K. pumila* and *K. foliosa* to 1.42 in *K. insignis* (Table 3). The values of these diversity parameters on the population level are in fact of the same order of magnitude as for the species level.

The expected heterozygosity (H_e) in the species ranges from 0.056 in *K. pumila* to 0.188 in *K. insignis*. At the population level, "fol-10" has the lowest and "ins-8" the highest value (Table 3).

Out of the 48 individual loci tested for deviation from Hardy-Weinberg equilibrium, six (12.5%) indicated significant difference ($p<0.05$) and found scattered in five populations. At the population level the mean of the inbreeding coefficient (F) is positive indicating a slight deficiency in heterozygosity though at individual locus level PGM-1 in "sch-14", AAT-2 in "ins-12" and "iso-1", and GPI-2 in "fol-13", "sch-5" and "sch-14" are negative indicating slight excess in heterozygosity (Table 4).

Table 3. Estimate of genetic variability within each population and species of genus *Kniphofia*.

Population	*P**	*A*	*AP*	A_e	H_o ± se	H_e ± se
fol-10	42.86	1.57	2.33	1.07	0.059± 0.033	0.058 ± 0.033
fol-13	57.14	1.71	2.25	1.11	0.082 ± 0.044	0.088 ± 0.044
K. foliosa	57.14	1.71	2.25	1.09	0.068 ± 0.037	0.070 ± 0.037
hild-4	28.60	1.43	2.50	1.18	0.106 ± 0.110	0.110 ± 0.071
ins-8	57.14	2.14	3.00	1.42	0.194 ± 0.107	0.187 ± 0.103
ins-12	57.14	2.00	3.00	1.39	0.171 ± 0.090	0.188 ± 0.097
K. insignis	57.14	2.14	3.00	1.42	0.181 ± 0.097	0.188 ± 0.100
iso-1	57.14	2.00	2.50	1.20	0.121 ± 0.056	0.134 ± 0.061
iso-2	57.14	1.86	2.50	1.28	0.151 ± 0.072	0.164 ± 0.081
K. isoetifolia	57.14	2.00	2.75	1.22	0.129 ± 0.059	0.143 ± 0.067
sch-5	42.86	2.00	3.30	1.27	0.146 ± 0.071	0.164 ± 0.080
sch-14	42.86	2.14	3.60	1.32	0.141 ± 0.077	0.172 ± 0.087
K. schimperi	42.86	2.14	3.60	1.30	0.143 ± 0.074	0.170 ± 0.084
pum-6	42.86	1.57	2.33	1.07	0.053 ± 0.035	0.056 ± 0.039

*P** is percentage of polymorphic loci at 95 % criterion; *A* is the mean number of alleles per locus; *AP* is the mean number of alleles per polymorphic locus, A_e is the effective number of alleles per locus; H_o is the observed heterozygosity; H_e is unbiased estimate of the heterozygosity expected under Hardy-Weinberg equilibrium; and se is standard error.

Table 4. Chi-square test for the deviation of the observed and expected allele frequencies from Hardy-Weinberg equilibrium at the polymorphic loci.

Pop.	PGM-1			PGM-2			AAT-2			GPI-2		
	χ^2	df	p	χ^2	df	p	χ^2	df	p	χ^2	df	p
fol-10	3.736	3	0.291	-	-	-	0.084	1	0.771	0.041	1	0.839
fol-13	1.546	3	0.672	0.000	1	1.000	0.059	1	0.808	4.288	1	0.038 *
hild-4	6.906	3	0.075	-	-	-	-	-	-	0.814	1	0.367
ins-8	7.495	6	0.278	0.053	3	0.997	0.017	1	0.896	2.715	3	0.438
ins-12	9.765	6	0.135	0.045	1	0.832	5.890	1	0.015**	3.948	3	0.267
iso-1	1.528	3	0.676	0.169	3	0.982	17.221	3	0.001**	0.754	1	0.385
iso-2	4.386	3	0.223	0.000	1	1.000	0.486	3	0.922	0.073	1	0.786
sch-5	12.196	6	0.058	-	-	-	1.126	3	0.771	13.538	3	0.004*
sch-14	37.331	10	0.000**	-	-	-	1.589	3	0.662	23.229	3	0.000**
pum-6	1.094	3	0.779	-	-	-	0.010	1	0.920	0.063	1	0.802

*p < 0.05; **p< 0.005 significant different between observed and expected allelic frequency.

The total genetic diversity at the polymorphic loci (H_T) in the species ranges from 0.123 in *K. foliosa* to 0.396 in *K. schimperi*. The proportion of H_T harboured within the population in *K. foliosa*, *K. insignis*, *K. isoetifolia*, and *K. schimperi* ranges from 99.5% in *K. schimperi* to 98.9% in *K. isoetifolia* indicating very low diversification between the populations (Table 5). At the generic level H_T ranges from 0.034 to 0.470 with a mean of 0.247. The genetic variation among the species (G_{ST}) is 6.6% (Table 6).

Table 5. Estimate of the F-statistics and unbiased Nei's genetic diversity statistics (1978) for the polymorphic loci.

Species	Locus	F_{IS}	F_{IT}	H_T	H_S	D_{ST}	G_{ST}
K. foliosa	PGM-1	-0.031	-0.027	0.264	0.263	0.001	0.004
	PGM-2	-0.018	-0.009	0.015	0.015	0.000	0.009
	AAT-2	-0.055	-0.055	0.100	0.099	0.000	0.000
	GPI-2	0.222	0.230	0.113	0.112	0.001	0.011
	Mean	0.022	0.027	0.123	0.122	0.001	0.006
K. hildebrandtii	PGM-1	0.199	------	0.398	------	------	------
	GPI-2	-0.165	------	0.373	------	------	------
	Mean	0.023	------	0.386	------	------	------
K. insignis	PGM-1	0.092	0.106	0.646	0.637	0.009	0.015
	PGM-2	-0.041	-0.036	0.087	0.087	0.000	0.005
	AAT-2	0.229	0.234	0.100	0.099	0.001	0.007
	GPI-2	-0.120	-0.120	0.481	0.481	0.000	0.000
	Mean	0.015	0.023	0.329	0.326	0.003	0.008
K. isoetifolia	PGM-1	0.129	0.134	0.451	0.448	0.003	0.006
	PGM-2	-0.032	-0.025	0.070	0.070	0.000	0.006
	AAT-2	0.030	0.031	0.176	0.176	0.000	0.001
	GPI-2	0.052	0.075	0.305	0.298	0.007	0.024
	Mean	0.078	0.088	0.250	0.248	0.003	0.011
K. schimperi	PGM-1	0.033	0.041	0.519	0.514	0.004	0.008
	AAT-2	0.085	0.085	0.277	0.277	0.000	0.001
	GPI-2	0.319	0.323	0.391	0.389	0.002	0.006
	Mean	0.141	0.146	0.396	0.393	0.002	0.005
K. pumila	PGM-1	0.073	-----	0.278	------	------	------
	AAT-2	-0.020	-----	0.039	------	------	------
	GPI-2	-0.041	-----	0.076	------	------	------
	Mean	0.042	-----	0.131	------	------	------

Table 6. Estimate of the F-statistics and unbiased Nei's genetic diversity statistics (1978) for the polymorphic loci at generic level.

Locus	F_{IS}	F_{IT}	H_T	H_S	D_{ST}	G_{ST}
PGM-1	0.094	0.154	0.470	0.440	0.030	0.064
PGM-2	-0.031	0.011	0.034	0.033	0.001	0.020
AAT-2	0.085	0.133	0.158	0.150	0.008	0.052
GPI-2	0.036	0.113	0.327	0.301	0.026	0.080
Mean	0.069	0.131	0.247	0.221	0.016	0.066

The similarity among the populations is higher than among the species as shown by Rogers (1972) similarity and unbiased Nei's (1978) genetic distance (Table 7). The similarity between the population ranges from 0.965 to 0.984 with a mean of 0.973. The lowest similarity is between populations of *K. isoetifolia* and the highest is between populations of *K. foliosa*. The largest genetic distance is between "fol-10" and "ins-12" (0.034, Table 7) with a mean of 0.011 that indicated very high genetic identity between the populations. Pair wise comparison of the species was made by pooling all genotypes to a species level and the species similarity coefficient ranges from 0.909 to 0.956 with mean of 0.939 and genetic distance ranges from 0.001 to 0.027 with mean 0.011. The smallest value is between *K. foliosa* and *K. pumila* and the highest value is between the *K. isoetifolia* and *K. schimperi*.

The phenogram of the unweighted pair group method with arithmetic average (UPGMA) clustering showed two major groups that depicted the relation among the species. The first grouping includes *K. foliosa, K. pumila* and *K. isoetifolia*. The second includes *K. hildebrandtii, K. schimperi* and *K. insignis* (Fig. 3). *K. insignis* appeared more divergent in the cluster than the rest.

Table 7. Rogers (1972) genetic similarity (above the diagonal), and Nei's (1978) unbiased genetic distance (below the diagonal) and mean of genetic identities (I* values): paired identities between each population and other populations.

pop.	fol-10	fol-13	hild-4	ins-8	ins-12	iso-1	iso-2	sch-5	sch-14	pum-6	I*
fol-10	------	0.984	0.940	0.911	0.900	0.948	0.922	0.931	0.917	0.980	0.987
fol-13	0.000	------	0.950	0.925	0.912	0.957	0.931	0.939	0.927	0.972	0.991
hild-4	0.009	0.005	------	0.943	0.928	0.942	0.935	0.957	0.954	0.954	0.992
ins-8	0.023	0.017	0.008	------	0.969	0.935	0.939	0.932	0.942	0.916	0.988
ins-12	0.034	0.027	0.014	0.002	------	0.918	0.926	0.928	0.941	0.902	0.983
iso-1	0.005	0.004	0.009	0.012	0.020	------	0.965	0.938	0.926	0.953	0.991
iso-2	0.015	0.013	0.013	0.008	0.014	0.003	------	0.939	0.932	0.929	0.989
sch-5	0.011	0.008	0.004	0.010	0.013	0.009	0.011	------	0.975	0.935	0.992
sch-14	0.018	0.013	0.006	0.007	0.008	0.013	0.014	0.001	------	0.92	0.990
pum-6	0.001	0.001	0.008	0.020	0.028	0.004	0.012	0.009	0.015	------	0.989

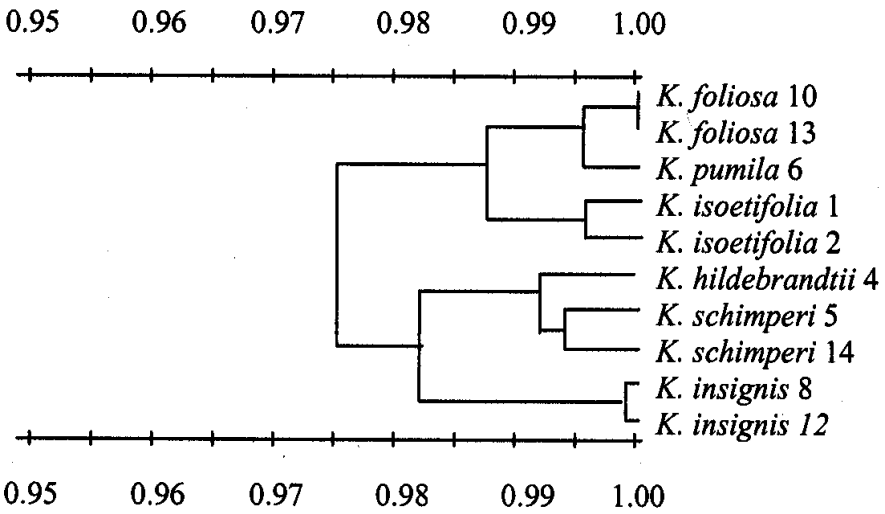


Fig. 3. Phenogram expressing overall level of genetic similarity among *Kniphofia* species based on Roger's (1972) coefficient of genetic similarity.

DISCUSSION

Variation within the populations

Four common parameters for the comparisons of intrapopulational variation are presented by Hamrick and Godt (1989), percentage of polymorphic loci (P), number of alleles per locus (A), effective number of alleles per locus (A_e), and expected heterozygosity (H_e). The mean value of P for plants (from data which were published until 1989) was calculated to be 34.2%. The analysed populations of genus *Kniphofia* all show a higher percentage of polymorphic loci, but here, it should be remarked that the number of loci and population is low, so the result may be biased. The indicated average value for A is 1.53. In our study all the populations scored higher in this parameter (from 1.57 to 2.14). When it comes to effective number of alleles, the average recorded by Hamrick and Godt (1989) is 1.15, and the populations of *K. insignis, K. schimperi, and K. isoetifolia* have a higher value. *K. hildebrandtii* scores close to the average, and the populations of *K. foliosa* and *K. pumila* scores below the average. The same relations are revealed by the expected heterozygosity (average for plants: 0.113), where again the populations of *K. insignis* showed the highest diversity (H_e= 0.187–0.188), and *K. pumila* and *K. foliosa* the lowest (H_e =0.056–0.058) diversity. It is striking that the only non-endemic species, *K. pumila* displayed the lowest diversity, but it should be remarked that only one population of this species was included in the analyses.

The endemic taxa, with an exception of *K. foliosa*, displayed more variation than expected, which indicates that they are not depleted of genetic variation at the population level. In the survey of Hamrick and Godt (1989) the expected heterozygosity that is particularly calculated for long-lived herbaceaous perennial taxa is H_e = 0.084 and for endemic taxa is H_e = 0.063, both attributes fitting to the endemic *Kniphofia* taxa.

The populations were mainly in Hardy-Weinberg equilibrium, and the few exceptions found were scattered both at locus and population. The outcrossing, probably by birds in most of the species, is accordingly rather efficient. The few significant deviations found showed a slight deficiency in the number of expected heterozygotes, which might indicate a slight inbreeding. The taxa might have a certain clonal vegetative growth leading to neighbours of the same genetic make up. This may further lead to consanguineous mating or Wahlund effect (Turner *et al*., 1982; Harada and Iwasa, 1996).

Variation among the populations

It was only possible to obtain material for more than one population for four of the species (*Kniphofia foliosa*, *K. insignis, K. isoetifolia* and *K. Schimperi*). In all cases the amount of variation that could be ascribed to the among population component was extremely low. It varied from 0.5% in *K. schimperi* to 1.1% in *K. isoetifolia*. Hamrick *et al*. (1991) have, in comparison, found that the average amount of variation among population in outcrossing plant species is about 10%. The same pattern is found when the genetic identities are compared. They show very high values ranging from 0.965 to 0.984 and the genetic distances are correspondingly low. As a whole the high genetic identity found between populations of all the species is predominantly the consequence of the distribution of the more common alleles and few rare alleles (Brown, 1990).

The most straightforward explanation for the low among population variation is gene flow. The conspecific populations are, however, situated with a far distance between them. Seed dispersal does not seem very probable under the conditions we found in the region today, and gene flow via pollen seems improbable given the disjunctive distribution. Even if we do no know the behaviour and flying distance between flower visits for the pollinators, the distance also in this respect appear to be a little too much.

The populations might represent fragments of a more continuous distribution in earlier times. In this case our result indicates that this fragmentation has taken place recently and that there has not been enough time to accumulate different alleles by mutation. Otherwise the populations are expected to lead to genetic drift and increased level of genetic divergence as the population size become smaller and gene flow decreases (Crawford, 1983).

Relation between the species

The analysed species show a strong genetic similarity, as shown by all the different analyses.

The amount of variation found among the species (G_{ST} = 6.6) is in fact lower than the expectation for among intraspecific populations of outcrossing species (=10%). This pattern is further supported by the values for the genetic identities among non-specific populations (ranging from I=0.90 to 0.98) and higher than the value of the genetic identify among congeneric species (I= 0.67) indicated in Crawford (1983).

When the taxa were clustered according to their genetic similarity, *K. foliosa* and *K. pumila* came closest. They are both characterised by very dense racemes and conspicuously exerted stamens. So this grouping is partially supported by the morphology of the taxa. *K. isoetifolia* clusters next to this pair. The latter share the special sequence of flower opening (from top to bottom) with *K. pumila*. Another species pair is constituted by *K. hildebrandtii* and *K. schimperi*, species that do not have very close morphological identity. The most deviating species, as depicted by the isoenzymes analysis is *K. insignis*. Morphologically it is the most deviating by its white flower colour (Sebsebe Demissew and Nordal, 1997).

The six studied species have resemblance in all vegetative characters and in fruits and seeds. They are predominately separated by traits, in the flower morphology and the inflorescence architecture, both particularly connected to pollination. The strange phenomenon of different species, in genetic respect more behaving as conspecific populations, indicates rather recent speciation (McClenaghan and Beauchmap, 1986). The results, therefore, indicate that all the Ethiopian K*niphofia* species share a fairly recent common ancestor, but have differentiated in floral and inflorescence character through rapid evolution due to selection by differential pollinator preferences. This hypothesis needs, however, further testing.

ACKNOWLEDGEMENTS

The authors thank the Ethiopian Petaloid Monocots project (NUFU-Ethiopia) for the financial grant, and Addis Ababa University and University of Oslo for providing the necessary facilities for accomplishment of the study.

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