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KARYOTYPE ANALYSIS OF ETHIOPIAN ENDEMIC *KNIPHOFIA* SPECIES

Tilahun Teklehaymanot[1,*], Endeshaw Bekele[1], Sebsebe Demissew[1] and Inger Nordal[2]

ABSTRACT: Image analysis was used to study the karyotype of six Ethiopian *Kniphofia* species: *K. foliosa*, *K. hildebrandtii*, *K. insignis*, *K. isoetifolia*, *K. schimperi* and *K. pumila.* The first five are endemic to Ethiopia. All have somatic chromosome number of 2n = 12, and follow the same karyotype formula: 1m + 3sm + 2st. There was significant variation among the species as to size and arm length of the chromosomes. However, the arm ratio (r) was not significantly different, indicating the centromeric position is constant among the species. The results indicated that the species (with the possible exception of *K. foliosa*) were closely related. The species differ morphologically, particularly, in flower size and shape, which obviously must be closely correlated to pollinator shape, size and activity. The evolutionary relationships among the taxa should further be studied using DNA sequences data from chloroplast and nuclear markers to elucidate the phylogenetic relationship of the *Kniphofia* species in Ethiopia.

Key words/phrases: Endemic, Ethiopia, Karyotype *Kniphofia*, Speciation

INTRODUCTION

The genus *Kniphofia* Moench (Asphodelaceae) comprises approximately 70 species, with major distribution in southern and eastern Africa. The plants are mainly bird-pollinated perennial herbs (Marais, 1973; Smith and Van Wyk, 1998). They are connected to wet habitats, but have otherwise a wide range from low savanna of about 900 m a.s.l. to montane and alpine forest of about 4400 m a.s.l (Ramdhani, 2006).

There are seven species in the Ethiopian flora: *Kniphofia foliosa* Hochst., *K. hildebrandtii* Cufod., *K. insignis* Rendle, *K. isoetifolia* Hochst., *K. pumila* (Ait.) Kunth, *K. schimperi* Baker, and *K. thomsonii* Baker. Local endemicity is a common feature in the genus, and out of the seven species, five are endemic to Ethiopia, i.e. *K. foliosa, K. hildebrandtii, K. insignis, K. isoetifolia,* and *K. schimperi* (Sebsebe and Nordal, 1997).

The studies conducted on the genus so far have shown that the species are diploid and the somatic cells have 2n = 12 chromosomes (Webber, 1932; de Wet, 1960). But *K. uvaris* has been reported with 2n = 13 and *K. snowdenii*

[1]Aklilu Lemma Institute of Pathobiology, Addis Ababa University, P.O. Box 56478, Addis Ababa, Ethiopia. E-mail: tilahunmt@yahoo.com
[2]Oslo University, P.O. Box 1066, Blindern, N-0316 Oslo, Norway.
*Author to whom all correspondence should be addressed.

with 2n=18 (Nayak and Sen, 1992).

de Wet (1960) showed, based on South African material, that the chromosomes may be categorized into three morphological groups: two pairs are significantly larger with secondary constriction, three pairs are medium-sized and one pair is short. The aim of the present study was to provide information on chromosome structure, particularly of the endemic Ethiopian species, which so far has not been studied, and to discuss the results in relation to morphological differentiation of the taxa.

MATERIALS AND METHODS

Seeds of *Kniphofia foliosa, K. hildebrandtii, K. insignis, K. isoetifolia, K. schimperi* and *K. pumila* were collected from nine localities in Ethiopia (Table 1). The seeds were sown in pots in the greenhouse of University of Oslo, Norway. Root tips, 2 - 4 cm long, were obtained from the plants and placed into cold water in glass vials. The vials were kept in a beaker with ice cubes and stored in a refrigerator (ca 0^0C) for an average of 24 hours, and fixed in 3 parts ethanol: 1 part glacial acetic acid for 2 hours (volume parts). They were then hydrolyzed in 1N HCl for 10 minutes at 60^0C. After a brief rinsing in water, the meristem was dissected out in a drop of 45% acetic acid on a slide and covered with a cover-slip. The cell wall was broken by gentle tapping. Aceto-orecin was used to stain the chromosomes by adding a drop at the edge of the cover glass. The slide was heated to spread the chromosomes and clear the cytoplasm. Permanent slide preparation was made by freezing and embedding the slide in Euparal.

Table 1. Localities, zones and regions in Ethiopia used as seed collection sites of the genus *Kniphofia* species

Species	Site of seed collection			Population number
	Locality	Zone	Region	
K. foliosa	Ali Doro	North Shewa	Oromia	10
	Dinsho	Bale	Oromia	13
K. hildebrandtii	Gedo	West Shewa	Oromia	4
K. insignis	Torban Ashie	North Shewa	Oromia	8
	Bullo Workie	East Shewa	Oromia	12
K. isoetifolia	Dinsho	Bale	Oromia	1
	Bekoji	Arsi	Oromia	2
K. schimperi	Goro Wonchi	West Shewa	Oromia	5
	Entoto	North Shewa	Oromia	14
K. pumila	Yirba Muda	Sidama	SNNP	6

The slides were analyzed using Zeiss Universal microscope with immersion lens 100 X /1.3 and ocular 10 X equipped with Optovar 2X for further magnification. The images of cell with spread chromosomes were captured with a Sony Video camera connected to Zeiss Universal microscope using NIH-image analysis program for Macintosh (National Institute of Health, Rasband, W. USA) on a Macintosh Power PC 5300 and saved. The phase-contrast image was analyzed using Adobe PhotoShop program (Adobe PhotoShop program, 1989 - 1998, USA.). The chromosomes total length and arm lengths were measured in micrometers with Scion Image program (NIH-image program modified for Window by Scion Corporation, 1998, USA) using the freehand measuring tool. The measurement was taken by leading the cursor along the chromosomes. It was possible to locate the centromeres by clicking in the active image window to zoom with the magnifying glass tool. The image printouts were generated from TIFF image files. The chromosomes were arranged according to their length. The arm length ratio was calculated. The relative arm length was obtained by the ratio of the total length of each chromosome and the total length of the longest chromosome for each species. The chromosomes are classified according to the nomenclature of Levan *et al*. (1964). Voucher specimens are deposited in the National Herbarium of Ethiopia, AAU.

One-way analysis of variance and Scheffe test were done using SPSS program (SPSS for Windows Release 8.00, SPSS, Inc., 1989- 1997) on total chromosome length, long arm length, short arm length and arm length ratio of homologous chromosomes. A hierarchical cluster analysis was undertaken using between group linkage and square Euclidean distance to analyze the relative similarity among the species based on the mean values of the chromosomal measurements.

RESULTS

All *Kniphofia* species considered in this study (Table 1) had 2n = 12 chromosomes. They were arranged in three size classes: large (L), medium (M), and small (S), though there was a fairly gradual transition from one size class to another (Fig. 1).

The image analysis gave a general karyotype: 1m + 3sm + 2st (Fig.1). The length of the homologous chromosome pairs (total length) differed significantly among the species ($p<0.01$) except for the short arms of chromosomes V and VI. The arm ratios for the homologous pairs were however not significantly different among the species, indicating that the centromere position was more or less fixed (Table 2).

Fig. 1. Karyotype of: (A) *Kniphofia foliosa*, (B) *K. hildebrandtii*, (C) *K. insignis*, (D) *K. isoetifolia*, (E) *K. schimperi* and (F) *K. pumila*

Table 2. The mean and standard deviation (SE) of total chromosome length (TL), long arm length (L), short arm length (S), arm ratio (r) and relative length (RL) of the chromosomes of the *Kniphofia* species in Ethiopia.

Species	Chromosome number	Total chromosome length (TL) Mean ± SE(µm)	Long arm length (L) Mean ± SE (µm)	Short arm length (S) Mean ± SE (µm)	Arm ratio (r) $L_{(mean)}$ / $S_{(mean)}$ Mean ± SE	Relative length
K. foliosa	I	32.68 ± 0.12	20.57 ± 0.39	12.09 ± 0.22	1.71 ± 0.06	1.00
	II	30.05 ± 0.25	19.30 ± 0.40	10.75 ± 0.41	1.81 ± 0.10	0.92
	III	27.11± 0.06	19.73 ± 0.14	7.38 ± 0.15	2.68 ± 0.07	0.83
	IV	26.43 ± 0.15	19.87 ± 0.13	6.56± 0.11	3.03 ± 0.06	0.81
	V	23.17 ± 0.15	16.07 ± 0.18	7.11 ± 0.29	2.28 ± 0.12	0.71
	VI	19.12 ± 0.24	15.02 ± 0.49	4.10 ± 0.49	3.96 ± 0.64	0.59
K. hildebrandtii	I	39.36 ± 0.13	24.77 ± 0.18	14.59 ± 0.18	1.70 ± 0.03	1.00
	II	36.22 ± 0.24	23.27 ± 0.28	12.95 ± 0.35	1.80 ± 0.07	0.92
	III	32.86 ± 0.21	23.90 ± 0.07	8.95 ± 0.21	2.68 ± 0.06	0.83
	IV	31.83 ± 0.30	23.95 ± 0.18	7.88 ± 0.22	3.05 ± 0.08	0.81
	V	28.45 ± 0.56	19.77 ± 0.12	8.68 + 0.50	2.31 ± 0.13	0.72
	VI	23.04 ± 0.32	18.38 ± 0.13	4.66 ± 0.26	3.99 ± 0.23	0.59
K. insignis	I	37.57 ± 0.61	23.59 ± 0.46	13.94 ± 0.30	1.70 ± 0.04	1.00
	II	34.64 ± 0.12	22.23 ± 0.02	12.40 ± 0.06	1.80 ± 0.06	0.92
	III	31.65 ± 0.23	23.02 ± 0.34	8.63± 0.20	2.67 ± 0.12	0.84
	IV	30.85 ± 0.36	23.04 ± 0.54	7.81 ± 0.18	3.04 ± 0.30	0.82
	V	26.93 ± 0.35	18.70 ± 0.35	8.24 ± 0.44	2.30± 0.16	0.72
	VI	21.98 ± 0.36	17.50 ± 0.07	4.48 ± 0.31	3.98 ± 0.29	0.58
K. isoetifolia	I	38.94 ± 0.39	24.57 ± 0.26	14.49 ± 0.53	1.71 ± 0.07	1.00
	II	35.30 ± 0.52	22.70 ± 0.39	12.60 ± 0.29	1.81 ± 0.05	0.91
	III	31.79 ± 0.34	23.06 ± 0.15	8.73 ± 0.46	2.67 ± 0.17	0.82
	IV	31.05 ± 0.43	23.30 ± 0.17	7.75 ± 0.33	3.03 ± 0.14	0.80
	V	27.18 ± 0.31	18.85 ± 0.23	8.33 ± 0.48	2.30 ± 0.16	0.70
	VI	22.39 ± 0.31	17.74 ± 0.52	4.65 ± 0.45	3.99 ± 0.49	0.58
K. schimperi	I	37.09 ± 0.19	23.31 ± 0.16	13.77 ± 0.27	1.70 ± 0.04	1.00
	II	34.01 ± 0.29	21.77 ± 0.13	12.24 ± 0.36	1.79 ± 0.06	0.92
	III	30.61 ± 0.15	22.16 ± 0.48	8.45 ± 0.51	2.67 ± 0.19	0.83
	IV	29.86 ± 0.22	22.41± 0.29	7.45 ± 0.36	3.04 ± 0.17	0.81
	V	26.16 ± 0.22	18.21 ± 0.07	7.96 ± 0.24	2.30 ± 0.07	0.71
	VI	21.60 ± 0.37	16.97 ± 0.31	4.63 ± 0.62	3.99 ± 0.63	0.58
K. pumila	I	36.10 ± 0.35	22.69 ± 0.29	13.27 ± 0.29	1.71 ± 0.03	1.00
	II	33.11 ± 0.12	21.31 ± 0.17	11.80 ± 0.11	1.81 ± 0.03	0.92
	III	30.49 ± 0.15	22.13 ± 0.21	8.36 ± 0.30	2.66 ± 0.12	0.84
	IV	29.92 ± 0.17	22.42 ± 0.29	7.50 ± 0.42	3.04 ± 0.22	0.83
	V	27.14 ± 0.69	18.04 ± 0.29	7.97 ± 0.33	2.29 ± 0.11	0.72
	VI	20.99 ± 0.29	16.87 ± 0.15	4.58 ± 0.68	3.99 ± 0.53	0.58

Chromosome I: The total chromosome length ranged from 32.34 to 39.82 µm with a mean of 36.77 µm ± 0.44 SE. The centromere was median. There was a terminal constriction on the long arm and a visible weak constriction on the short arm. The mean difference of the total chromosome, long arm and short arm lengths of *K. foliosa* were different from the rest ($p < 0.01$), but the short arm length was not significantly different from that of *K. schimperi* and *K. pumila* ($p > 0.01$).

Chromosome II: The total chromosome length ranged from 28.86 to 37.03 µm and with a mean of 33.70 µm ± 0.39 SE. The centromere was submedian. There was a terminal constriction on the long arm. At the terminal position of the short arm there was a knob in all, but in *K. insignis*, it appeared as satellite. The mean difference of the total length of chromosome of *K. foliosa* differs significantly from the rest ($p < 0.01$), the difference was however, not significant among *K. hildebrandtii*, *K. insignis* and *K. isoetifolia* and between *K. schimperi* and *K. pumila* ($p > 0.01$). The mean difference of long arm length of *K. foliosa* and *K. schimperi* was significantly different from all ($p < 0.01$). The mean difference of short arm length of *K. foliosa* was significantly different from that of *K. hildebrandtii* ($p < 0.01$).

Chromosome III: The total chromosome length ranged from 26.72 to 33.50 µm with a mean of 30.55 µm ± 0.35 SE. The centromere was submedian. There was constriction on the long arm and a satellite on the short arm in *K. insignis* and *K. isoetifolia*. The rest had a knob-like structure at the terminal position on the short arm. All seem to have an additional terminal constriction on the short arm. The variation among the species was similar to chromosome II, but *K. pumila* was significantly different from all ($p < 0.01$).

Chromosome IV: The total chromosome length ranged from 26.09 to 32.83 µm with a mean of 28.98 µm ± 0.44 SE. The centromere was subterminal. All had a terminal constriction on the long arm; satellites were lacking. The mean difference of total chromosome length of *K. foliosa* and *K. hildebrandtii*, the long arm lengths of *K insignis* and the short arm length of *K. hildebrandtii* was significantly different from all the species ($p < 0.01$).

Chromosome V: The total chromosome length ranged from 22.82 to 30.41 µm and with a mean of 26.63 µm ± 0.33 SE. The centromere was submedian. It has a terminal constriction on the long arm. The short arm in *K. hildebrandtii* and *K. insignis* has a satellite, and a terminal knob is visible on the short arms of *K. foliosa* and *K. pumila*. The mean difference of the total chromosome length of *K. hildebrandtii* was significantly different from all the species ($p<0.01$), and so was *K. foliosa* from *K. isoetifolia* and *K. pumila*. Also the long arm length of *K. foliosa* was significantly different from all the species except in relation to *K. insignis*. The short arm length was, however, not significantly different among the species ($p > 0.01$).

Chromosome VI: The total length ranges from 18.51 to 24.96 µm and with a mean of 22.30 µm ± 0.25 SE. The centromere was subterminal. There was

a terminal constriction on the long arm of all species and satellite or a terminal knob on the short arm of *K. hildebrandtii, K. insignis* and *K. pumila*. The mean difference of total and long arm length of *K. foliosa* was significantly different from all the species except with *K. pumila* ($p < 0.01$).

The mean values of the chromosomal measurements from the data matrix (Table 2) were used to construct a phenogram (Fig. 2). *Kniphofia foliosa* was the most diverging. It was seen that *K. insignis* and *K. isoetifolia* were closely related as to karyotype morphology, and the same was true for *K. schimperi* and *K. pumila.* The last species, *K. hildebrandtii*, clustered with the former two.

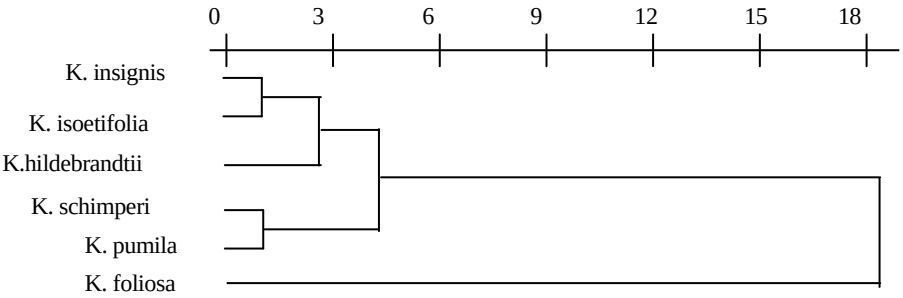

Fig. 2. Phenogram showing the relation among the six analyzed *Kniphofia* species on karyotype differentiation.

DISCUSSION

The evolutionary differentiation of the *Kniphofia* species in Ethiopia has taken place without involving either changes in ploidy levels, aneuploid numbers or strong karyotypic differentiation. The conclusion of de Wet (1960) based on South African material: "Species evolution of *Kniphofia* must have taken place through point mutations and small chromosomal aberrations" is accordingly supported by this study.

The image analysis of the somatic chromosomes of the six *Kniphofia* species agrees with the studies of Webber (1932) and de Wet (1960), although there are some differences on the centromeric positions. de Wet (1960) found chromosome VI to be submedian in the South African taxa, whereas it is subterminal in the Ethiopian taxa. Webber (1932) found subterminal centromeres in chromosome number III, whereas it was submedian in our material.

The karyotypic analyses indicated that the Ethiopian taxa are closely related among themselves, with *K. foliosa* as the most distinct species. Regarding the *Kniphofia* species in Ethiopia, a numerical analysis has never been undertaken based on morphological data, neither phenetical nor cladistical, so there are no hypotheses on the relationship among the taxa to be tested by the new data. The work, so far, undertaken was only using few samples of *Kniphofia* species in Ethiopia (Ramdhani, 2006), though morphologically the species differ particularly, in flower size and shape, which obviously must be closely correlated to pollinator shape, size and activity. It is reasonable to believe that such traits are under strong selection, leading to rapid speciation, whereas other traits, including karyotypes, may remain more stable.

The deviating *K. foliosa* differs from all the others by being very robust, almost arborescent with a stem of up to 40 cm, and is thus morphologically distinct. The relatively remote position in the phenogram by this species may represent the true relationship among the taxa. All the other species are slender and herbaceous plants. The indicated species pair, *K. insignis* and *K. isoetifolia* differ between themselves particularly by the opening sequence of the inflorescence, the youngest stages at the top in the former and at the bottom in the latter. This difference was also present in the other pair, where *K. pumila* share the trait of unusual opening sequence with *K. isoetifolia*, whereas *K. schimperi* has the ordinary opening sequence as found in *K. insignis*. If the clustering based on karyotypes shows the true genetic relation, then the particular flower sequence of *K. isoetifolia* and *K. pumila* must have originated independently (Sebsebe Demissew and Nordal, 1997). The evolutionary relationships among the taxa should further be studied using DNA sequence: data from chloroplast and nuclear markers to elucidate the phylogenetic relationship of the *Kniphofia* species in Ethiopia.

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