

CYTOLOGICAL STUDIES IN *GIRARDINIA DIVERSIFOLIA* (LINK) FRIIS

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Abstract

Karyomorphological and micromorphological observations of different collections of the species *Girardinia diversifolia* show enough evidences to the existence of 2 or more ecotypes in the species.

Introduction

Girardinia diversifolia (Link) Friis. (Urticaceae), commonly known as "Himalayan nettle" is widely distributed in the sub-tropical and temperate Himalayas. It is known to occur in India from Kashmir to Sikkim upto 2,100 m and extending to the Khasia hills in the eastern part and from Marwar, central India to Travancore in the south (Hooker 1872-1897). It occurs in China from the central part to the east. It is also distributed in Nepal, Bhutan, Burma, Malaysia, Java and Sri Lanka. In Nepal, it is distributed from the east to west in the altitudinal range of 1,200-3,000 m. The main ethnic groups engaged in exploiting this plant are the Rais, Sherpas, Magars, Gurungs etc. These people have Mongoloid features, different languages and Indo-Tibetan cultures. It is an indigenous fiber yielding plant of Nepal. The nettle fiber has been used in hills for domestic purposes for a long time. In the past few years, there has been growing interest among the rural communities on wider exploitation of this fiber resource and development of a wide variety of woven products for commercial purposes.

Comparative studies of the external morphology of the bracts, seed, cotyledonary leaves, seedling behavior, fibers and amino acid contents of leaves and seeds of 2 types of *G. diversifolia* from different localities were made by Sindh & Shrestha (1985). The present report describes the karyomorphological and micromorphological studies in this taxon to provide enough evidences to the earlier work on the existence of 2 or more ecotypes in the species.

Materials and Methods

Seed samples Nos.92001K, 92006M, 92001C, 92005J, 92001D and 92001B were respectively collected from different places of Kathmandu (1,800 MSL), Mude (1,500 MSL), Charikot (2,500 MSL), Jiri (2,200 MSL), Jiri Local Development Centre Compound (2,200 MSL) and Bhedetar (1,400 MSL). Somatic chromosomes study was carried out by pretreating healthy root tips as well as leaf tips in 0.002 M 8-hydroxyquinoline for 3 h at 10-15°C and fixed in ethyl alcohol: glacial and acetic acid (3:1). Leaf tips were fixed in ethyl alcohol, pure chloroform and glacial acetic acid (6:3:1) mixture. For long time preservation, they were stored in 70% ethyl alcohol at 4°C. The

materials were then washed with 45% acetic acid and stained with 2% aceto-orcein and 1 N HCl (9:1) for 3 h or more in a few cases. Squashes were made in 45% acetic acid. Observation was made and best plates were selected for drawing and photomicrography. Drawings were made at table level using Opticlite 1366 Camera Lucida apparatus with built in polarized filter using 10 X eye piece and 1.30 FL 100 X oil immersion objective and later enlarged to a suitable size. For photomicrography of cytological preparations, low speed (ASA 32) high contrast films were used. The chromosome measurements were made from the drawn figures. Detailed karyomorphological study of this taxon has been made following the nomenclature of chromosomes proposed by Levan *et al.*, (1965). For micromorphological observations of hairs of different collections, above mentioned methods were applied.

Results and Discussion

Karyotype analysis of 2 collections (92001K and 92005J) of *G. diversifolia* are given below:

Collection No.92001 K: Somatic chromosome number and karyotype were determined from young root tips as well as leaf tips. The chromosomes are $2n = 20$, small and more or less homogeneous in size (Fig.1). The karyotype formula is $2n = 20 = M_8 + m_8 + sm_2 + st_2$. The karyotype of this taxon comprises 4 types of chromosomes. Four pairs of chromosomes with centromere at median points from the first type, 4 pairs with centromere at median regions represented the second type. Third type of chromosomes represented by a single pair bearing centromere at the submedian region, and one pair of chromosomes with centromere at sub-terminal region forms the fourth type. The chromosome length of a genome ranges from 0.7 to 1.7 μm with an absolute length of 9.4 μm (Table 1).

Table 1. Measurements of chromosomes of *Girardinia diversifolia* (92001K).

Chromosome pairs	Long arm (μm)	Short arm (μm)	Total length (μm)	r-value (l/s)	Relative length	Centromere position
I	1.3	0.4	1.7	3.25	17.89	st
II	0.7	0.4	1.1	1.75	11.58	sm
III	0.6	0.4	1.0	1.50	10.54	m
IV	0.5	0.4	0.9	1.25	9.47	m
V	0.5	0.4	0.9	1.25	9.47	m
VI	0.4	0.4	0.8	1.00	8.42	M
VII	0.4	0.4	0.8	1.00	8.42	M
VIII	0.4	0.4	0.8	1.00	8.42	M
IX	0.4	0.4	0.8	1.00	8.42	M
X	0.4	0.3	0.7	1.33	7.38	m

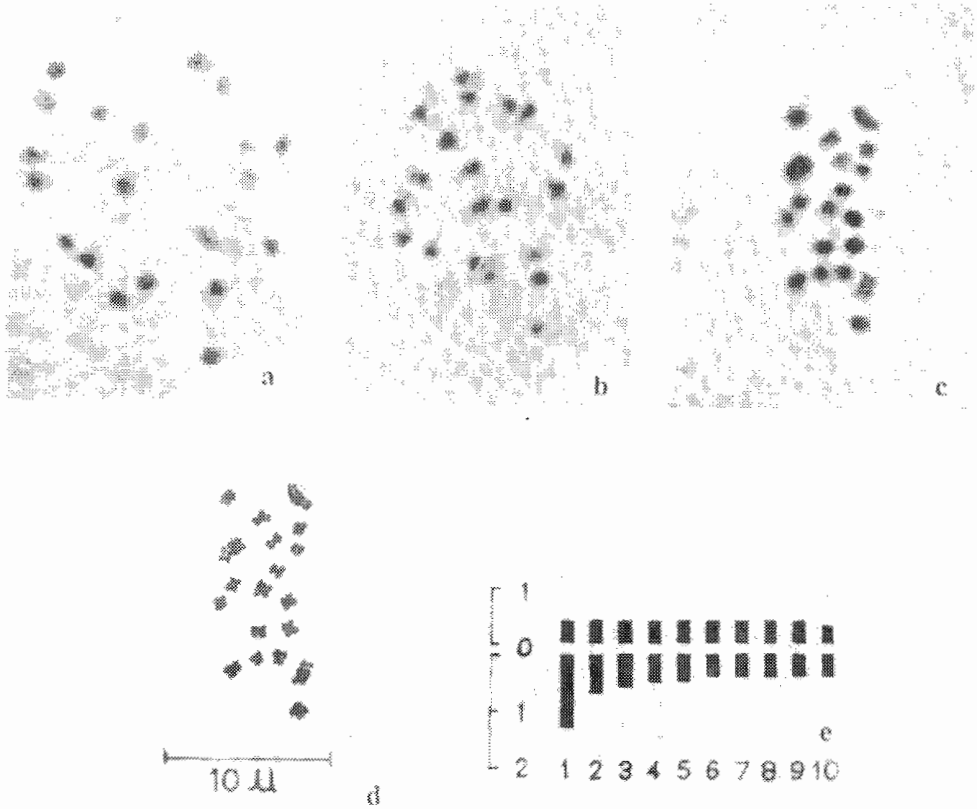


Fig.1. Karyomorphological observations on *Girardinia diversifolia* 92001 K.
 a,b,c. Photomicrographs of somatic metaphase plate (2n=20) x 2500.
 d. Camera Lucida drawing of somatic metaphase.
 e. Idiogram in 1 unit = 1 μm.

Collection No.92005J: Somatic chromosome number of this collection was determined by frequency counts. The most common number recorded from the root is $2n=20$ (Fig.2). The karyotype formula is $2n=20 = M_4 + m_{14} + sm_2$. There are 3 types of chromosomes in this collection. The first type has 2 pairs of chromosomes having centromere at their median points. Second type has 7 pairs of chromosomes having centromere at the median regions and a pair of chromosomes with submedian centromeres forms the third type. The chromosome length of a genome varies from 0.8 to 1.3 μm with an absolute length of 9.8 μm (Table 2).

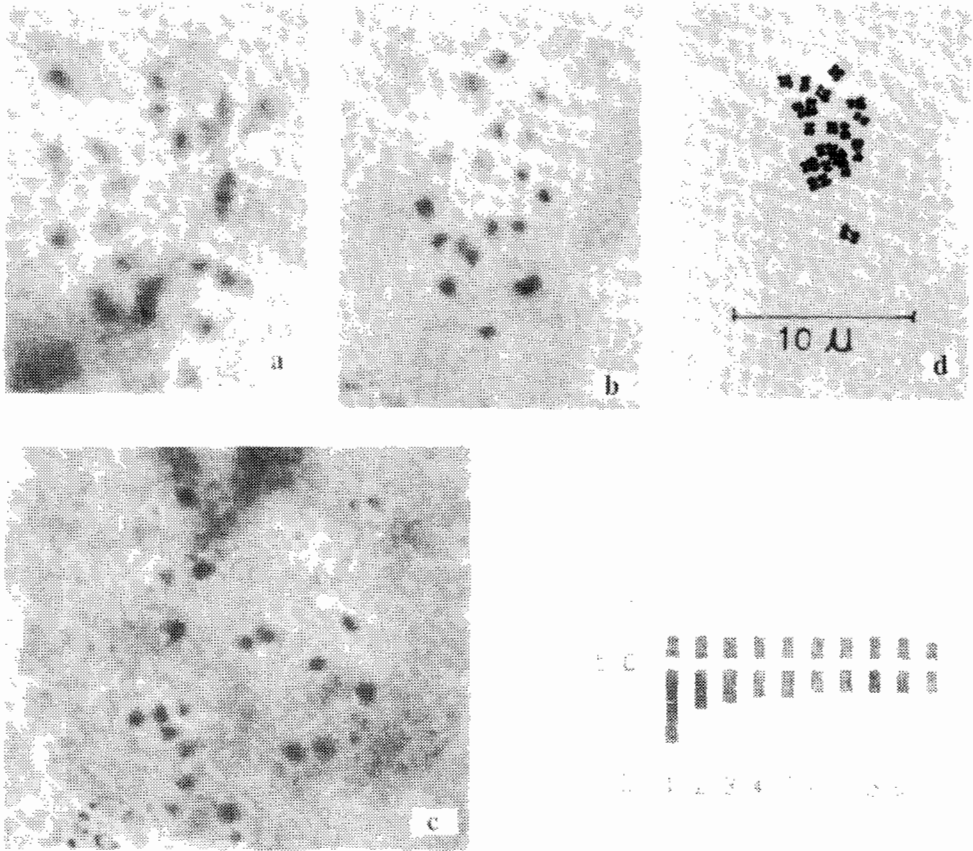


Fig.2. Karyomorphological observations on *Girardinia diversifolia* 92005 J.

a,b,c. Photomicrographs of somatic metaphase plate ($2n=20$) x 2500.

d. Camera Lucida drawing of somatic metaphase.

e. Idiogram in 1 unit = $1 \mu\text{m}$.

The chromosomes are smaller and more or less homogeneous. The average length of chromosomes is $0.95 \mu\text{m}$ in 92001 K and $0.98 \mu\text{m}$ in 92005J. The ratio of smallest and largest chromosomes ranged from $1.63 \mu\text{m}$ in 92005J to $2.48 \mu\text{m}$ in 92001 K. The karyotype data of the taxa show uniform features on the basic structure. However, a few chromosomes with submedian and subterminal centromeres are also reported. The taxa have slightly graded karyotype.

The micromorphological studies on the trichomes (Figs.3 & 4) indicate the existence of 2 different ecotypes also strengthened by earlier studies of Singh & Shrestha (1989) on the differences in the hairyness, bract size, seed characteristics, cotyledonal size, fibre size yield and amino acid contents. The differences exhibited by the populations of *G. diversifolia* growing in two different localities indicate the effect of different

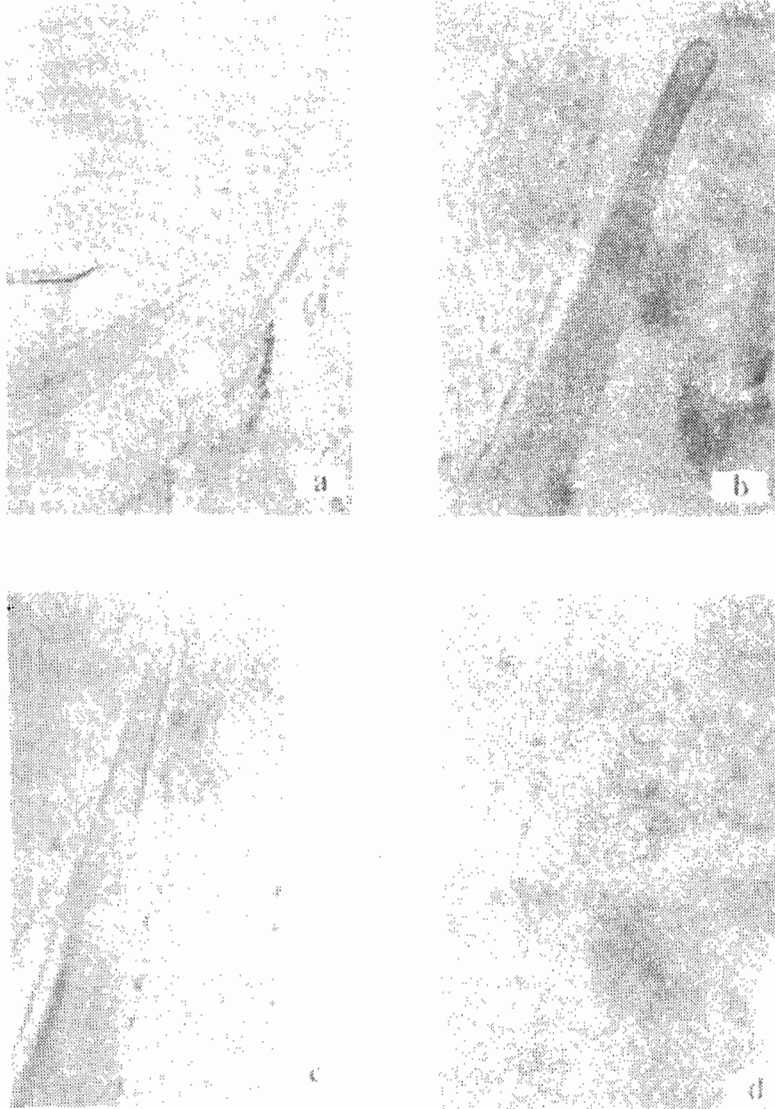


Fig.3. Micromorphological observations on *Girardinia diversifolia* 92001 x.
a. Stinging hairs from young leaf x 100.
b. Stinging hairs with densely warty cell wall with rounded tip having small pore x 130.
c. Stinging hairs with smooth and highly warty cell wall with sharply pointed tips x 100
d. Glandular hairs with bi-celled cap from young leaf x 300.

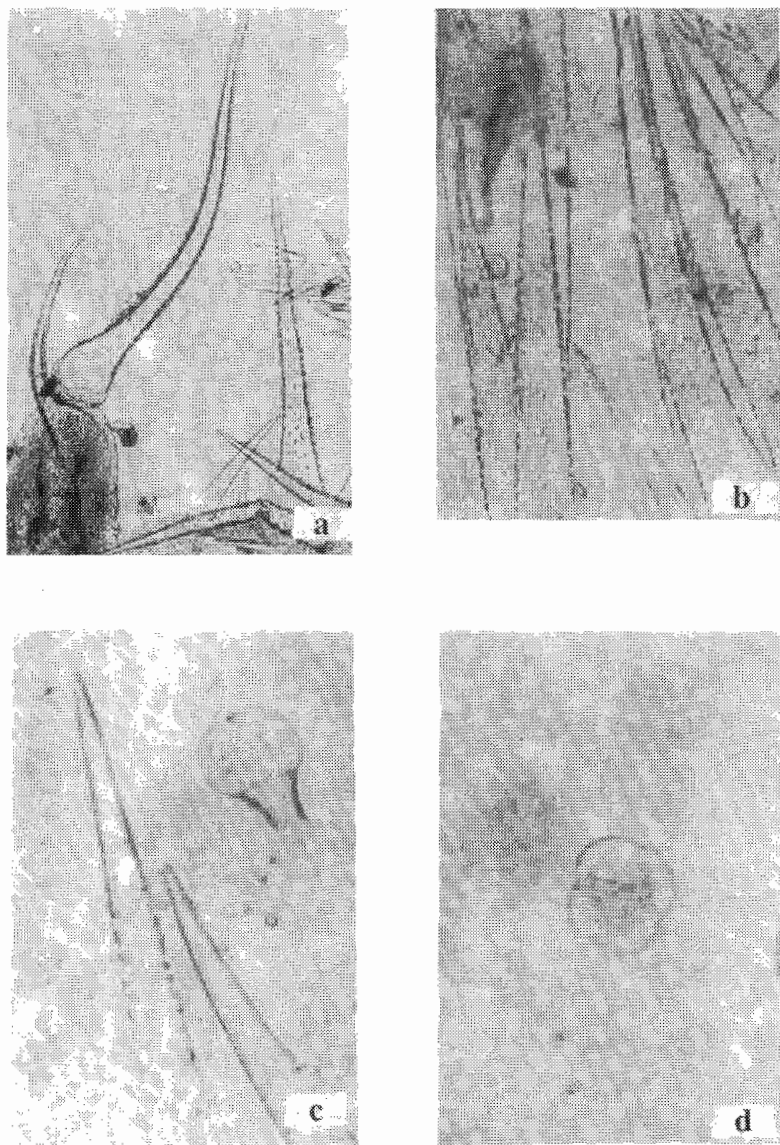


Fig.4. Micromorphological observations on *Girardinia diversifolia* 92005 J.

- a. Two different types of hairs from young leaf x 70.
- b. Stinging hairs with densely warted cell wall in more population x 100.
- c. Stinging and glandular x 100.
- d. Head of glandular hairs (Upper view) x 300.

Table 2. Measurements of chromosomes of *Girardinia diversifolia* (92005J).

Chromosome pairs	Long arm (μm)	Short arm (μm)	Total length (μm)	r-value (l/s)	Relative length	Centromere position
I	0.7	0.6	1.3	1.16	13.27	m
II	0.7	0.5	1.2	1.40	12.23	m
III	0.7	0.4	1.1	1.75	11.23	sm
IV	0.6	0.4	1.0	1.50	10.21	m
V	0.5	0.4	0.9	1.25	9.18	m
VI	0.5	0.4	0.9	1.25	9.18	m
VII	0.5	0.4	0.9	1.25	9.18	m
VIII	0.5	0.4	0.9	1.25	9.18	m
IX	0.4	0.4	0.8	1.00	8.17	M
X	0.4	0.4	0.8	1.00	8.17	M

ecological situations. The karyomorphological and micromorphological observations of two collections viz., 92001 K and 92005 J show the differences making evidences of existence of two ecotypes. The structural changes touch mainly the medianly constricted component of the primary karyotype. The two collections can thus be called as two different ecotypes of the species *G. diversifolia*.

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