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Phytochemical and Chromosome study of *Gloriosa superba* L. (Liliaceae)

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Abstract:

Gloriosa superba L. is important medicinal plant species containing as well as having some ornamentals and horticulture values. The plant was under threatened category due to its impulsive harvesting from wild as it widely used by medicinal industries for its colchicine content. It is available during West Bengal with high variability in morphological and colchicine content. The present study on *Gloriosa superba* L. agreements with detailed comparative study of somatic chromosome of six population of West Bengal. In view of this species as well as submission of cytological races have been already experimental in this species and a detailed karyotype analysis which were found to be diploid in all the inhabitants. The present investigation shown that the 2n= 24 chromosomes are in depth of this species. The karyomorphological variations in different population may be due to the minute structural changes of chromosomes.

Keywords: *Gloriosa superba*. Different population. Karyotype. Somatic chromosome.

1. Introduction:

The world is moving towards the natural medicine due to the absence of any side effects in its application. Medicinal plant has got importance in this scenario and the production and marketing of these medicinal plants are confined to specific locations. One such is a very important medicinal plant is *Gloriosa superba* L. The tuber explant of this herb is the main source of colchicine and it has got much importance in Indian's export market and earns more foreign exchange. Alkaloids extracted from this plant are used in pharmaceutical in large (Chatterjee and Ghosh, 2015; Basu & Jha, 2011).

Gloriosa superba L is among some of the modern medicines most important plants actually facing local extinction (Dhushara, 2004). Its name *Gloriosa* from the word "Gloriosus", which means handsome and *superba* from the word "superb' means splendid or majestic kind. This plant has been source of medicine right from the ancient time. *Gloriosa superba* L. is a perennial tuberous climbing herb is widely distributed in tropical and subtropical parts of India including foothills of Himalayas (Vijayayalli and Mathew, 1990).

Although protocols for cytogenetic analysis are available of *Gloriosa superba* L. but in the present study focus on a detailed cytological study may be able to reveal the structure and dynamics the populations, the mechanisms of the maintenance of various cytotype and the intraspecific relationship occurring over the wide geographical areas (sharma and Sharma, 1980).

Plant genetic resources support the live hoods of every person on the Earth. Whether used by farmers or by plant breeders, plant genetic resources are a reservoir of genetic adaptability that acts as a buffer against potentially harmful environmental and economic change. However, genetic erosion is occurring all around the world at an alarming rate due to changes in land use, rising population pressure and industrial development (Yadav *et al*., 2013).

2. Botanical Description:

There are several associated species under genus *Gloriosa*. It is native tropical Asia and Africa. It is found growing thought out tropical India, from North- West Himalayas to Assam and all along the Western Ghats in Karnataka (Kavina *et al,* 2011). It contains alkaloids like colchicine ($C_{22}H_{25}O_6N$) and its derivative like gloriosin and colchicocide ($C_{27}H_{33}O_{11}N$) along with Benzoic acid, Salicylic acid, sterols and resinous substances and therefore, the demand of this plant is increasing day by day (Ade and Rai, 2011).

Gloriosa superba L. is a perennial creeper with hollow stem of about 6m. The leaves are etiolated, alternated, sessile, lanceolate and spear shaped with curved end. It has wavy edged greenish to yellow and red flowers are large, solitary at ends of branches, six perianth lobes are bent backwards, six radiating anthers and the styles are bent also. Fruits are oblong, ellipsoid capsule. Seeds are numerous and rounded (Jana and Shekhawat, 2011). Stems arise from a single V- shaped fleshy cylindrical tuber.

3. Materials and Method:

- **Collection of plant material:**

Tubers of *Gloriosa superba* L. of the same age (1 year old) were collected from six different populations of West Bengal: Rahara,North 24 Parganas (I); Narendrapur, South 24 Parganas (II); Sargachi, Murshidabad (III); Kalyani, Nadia (IV); Benapur, Medinipur (V); Lava, Darjeeling (VI). The tubers were planted and maintained in the Experimental garden of the Department of Botany, RKMVC College, Rahara and tubers or other plant parts were procured from these stocks.

- **Morphological study of different populations:**

The tubers planted in the Experimental Garden sprouted in the month of June. The morphological characteristics of the plants were assessed taking into consideration traits like leaf length, leaf width, intermodal length, tuber size, diameter of the tubers, flower color and length of petals. Data was taken from randomly selected ten plants from each population of plant collected.

- **Extraction of Colchicine from tubers:**

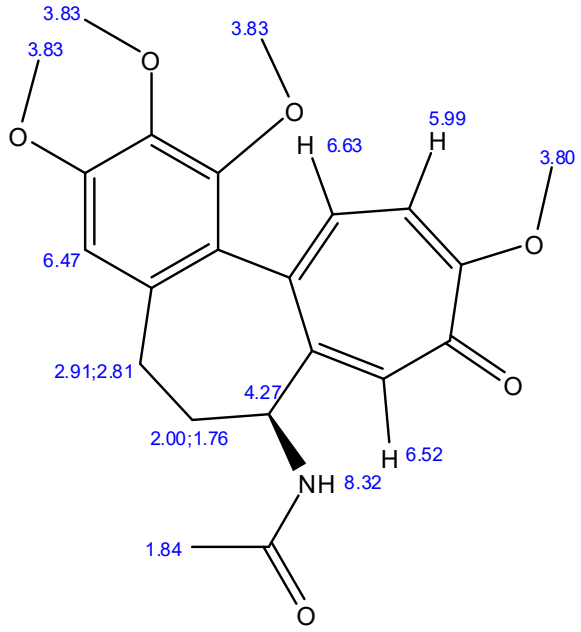
Tubers of *Gloriosa superba* L. collected from six different populations of West Bengal were qualitatively assessed for the presence of colchicine. For this purpose, ten randomly chosen plants of each population were taken. Colchicine was extracted and analyzed. For extraction, tubers were washed thoroughly by running tap water and dried in the sun for 10 days and again dried at 40°C in a hot air oven for 24 hours. Dried tubers were sliced in small pieces and powdered by electronic mill. Approximately 50 gm of sample were taken into a thimble and placed in a soxhlet apparatus, were set up with methanol (B.P = 65°C) solvent. The extraction was carried out for 16 hours to ensure the complete extraction of colchicine from the dried tubers of *Gloriosa superba* L. After completion of extraction the dark brown extract was then cooled, filtered (Watman, Grade No.-1), concentrated using rotary evaporator, and finally by vacuum suction to get a crude dried extract which was black yellow in color. Weighed accurately 8 gm sample and dissolved in 80ml methanol and finally made up to 1mg/ml, filtered the sample through 0.2µm membrane filter before analysis. The extract was evaporated on a water bath and the residue was redissolved in methanol. The methanolic extraction was used for qualitative and quantitative analysis.

- **Standard preparation:**

A stock solution of standard Colchicine was prepared in micro centrifuge tube (Tarson) by dissolving 10 mg of accurate weighed colchicine standard and dissolved in 1 ml of methanol and finally making the volume up to mark with solvent and prepare solution 1 mg/ml.

➢ **NMR Analysis of standard Colchicine:**

The ^{1}H NMR spectra were measured using JEOL ECS 400 MHz spectrometer by dissolving the compound (~3 mg) in 0.6 mL DMSO- d_6 at room temperature. The chemical shifts are reported in parts per million (ppm).



Structure of Colchicine

➢ **Chemicals material:**

All solvents and chemicals used were obtained from E-Merck, India for HPTLC analysis i.e., Acetic acid, Methanol, Chloroform, Acetonitrile, Acetone and Diethylamin. The reference standard of Colchicine was purchased from Himedia® (Batch No PTC1302- 1G).

➢ **Qualitative and quantitative analysis of colchicine:**

For qualitative analysis of colchicine in the samples, the methanolic extract was concentrated and subjected to TLC. The analysis of extracted colchicine is done with thin layer chromatographic technique. TLC plates (20×10 cm) coated with layers of silica gel 60 F254 (Merck, India, Batch no. HX245754) was used. These spot are applied at 20 mm distance and allow drying. Chloroform: Acetone: Diethylamine (5:4:1) V/V was taken as mobile phase in development chamber and allowed to saturate for 20 min. One major band which was detected visually and under UV 353 nm in the plate of colchicine extract. The distance travelled by Colchicine was 8 cm while solvent was up to 8 cm. The amount of berberine was measured with calibration curve of standard Colchicine.

The major compounds present in the crude mixture of stems of *Gloriosa superba* L., extract were also analyzed and quantified by High performance liquid chromatography (Waters 600/2998), briefly, a Waters Nova – Pak ® C18 (3.9 mm_300 mm, 4 uM particle). As a mobile phase water (A): acetonitrile (B) with 0.1% acetic acid was taken. A 20 µL crude mixture dissolved in methanol was injected and identified with standard being detected at 254 nm wavelength, using uv detector. The flow rate was kept at 1 ml/min. The gradient was set as starting at 92% solvent A and ramping to 75% solvent A in 5 min, holding at 75% solvent A for 2 min, ramping again to 100% solvent B in 10th min and finally backing to 92% solvent A in next 9 min. The concentration of compound colchicine was calculated from the area of the standard injected at the same parameters.

➢ **Cytological analysis of different populations:**

Root tips of ten randomly selected plants of each population were cytological analyzed to ascertain the cytological status of six different populations. Root tips were pre- treated in p-dichlorobenzene for 8 hours at 17ºC temperature and fixed overnight in acetic acid and ethanol mixture (1: 3) at 4 º C temperatures and stained with 2% aceto orcin (w/v): HCl mixture (9:1). The tissues were squashed with 45% acetic acid and observed under light microscope. The mitotic metaphase plates were photographed with Leica R M32 camera and the positive prints of the plates were used to prepare karyotypes. Karyomorphology were analyzed by measuring chromosomes on photographs at 2000x final magnification following the nomenclature of Levan *et a*l, 1964. For constructing karyotype formula, chromosomes were classified under five groups- A, B, C, D and E. At least ten individuals per population were scored, for each individual at least ten metaphases were analyzed.

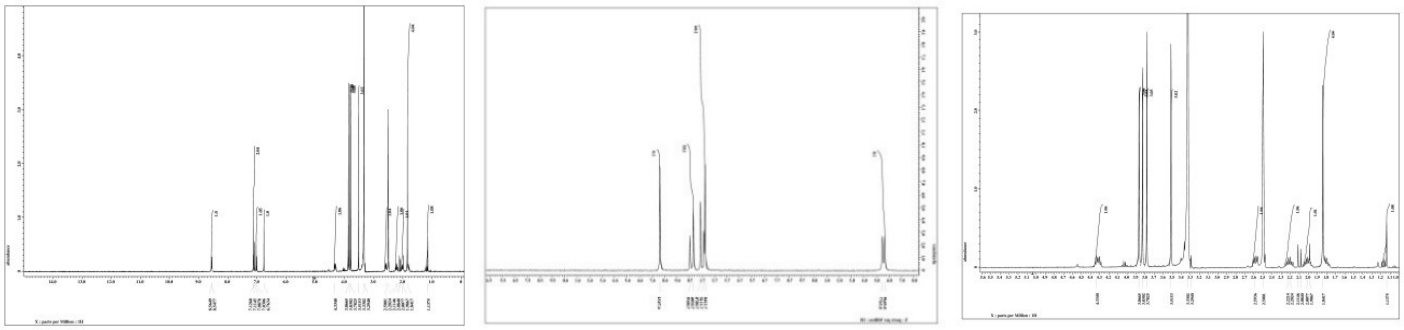
4. Results and Discussion:

❖ **NMR Analysis of standard Colchicine:**

^{1}H-NMR (DMSO-d_6, 400 MHz) δ (ppm): 8.55 (d, 1H), 7.12 (s, 1H), 7.10 (d, 1H), 7.02 (d, 1H), 6.76 (s, 1H), 4.33 (m, 1H), 3.87 (s, 3H), 3.83 (s, 3H), 3.78 (s, 3H), 3.51 (s, 3H), 2.59 (m, 1H), 2.20 (m, 1H), 2.00 (s, 1H), 1.84 (s, 1H), 1.80 (m, 3H), 1.13 (s, 1H).

❖ **Resonance assignments:**

The structure of berberine was verified by one-dimensional proton and carbon NMR spectra combined with 1H NMR and JEOL ECS 400 MHz spectrometer. Since no additional peaks were observed, we assume that no stereoisomers were present in this sample. All carbon and proton resonance of berberine was easily assigned from the combined use of HMBC (Figure 2). Berberine consists of five rings and two methoxy groups at positions 19 and 20.



❖ **Collection of plant material:**

The harvest season of *Gloriosa superba* L. is June for all the populations collected in the present study. The plants were collected from the six different populations of six different districts of West Bengal. The altitude ranged from 4to 2134 meters above the sea level and temperatures during the harvesting time ranged from 22 to 35ºC. The details geographical distributions of the five populations are of collections are summarized in Table- 1.

Table-1: Geographical details of the six populations of *Gloriosa superba* L.

Sl. No.	Population	Location	Soil types	Altitude	Temperature during harvesting	Geographical details
1.	I	Rahara (North 24 Parganas, West Bengal)	Loamy soil	16 meters above the sea level	35ºC	Latitude- 22.72°N Longitude- 88.38°E

2.	II	Narendrapur (South 24 Parganas, West Bengal)	Sandy Loam	4 meters above the sea level	32ºC	Latitude- 22°11'6"N Longitude- 88°20"E
3.	III	Sargachi (Murshidabad, West Bengal)	Slightly acidic, mainly gengetic alluvial soil	19 meters above the sea level	27ºC	Latitude- 19°18'N Longitude- 84°51' E
4.	IV	Kalyani (Nadia, West Bengal)	Gengetic alluvium, light texture	7.8 meters above the sea level	30ºC	Latitude- 22°53'N Longitude- 88°9' E
5.	V	Benapur (Medinipur, West Bengal)	Sandy Loam	23 meters above the sea level	32ºC	Latitude- 22.4333°N Longitude- 87.3333° E
6.	VI	Lava (Darjeeling, West Bengal)	Predominantly reddish in color mainly mixed sandy loam.	2134 meters above the sea level	22ºC	Latitude- 27.0500°N Longitude- 88.2667°E

❖ **Morphological variations in different populations:**

Variations in morphology were study in the plants of six collected populations. The plants of population-VI were distinguishable different from the plants of other five populations and population- I was also distinguishable from other populations. The plants of population-II, III, IV and V were morphologically related. Details morphological characters and measurements (leaf length, leaf width, intermodal length, tuber size, diameter of tubers flower color and length of petals) are summarized in Table-2. Measurements of 1 year old tuber were taken for analysis, only mature leaves and mature flowers were measured to determine leaf length, leaf width, internodal length and flower color, length of petals for this study. Plants of population VI were smallest than other populations but tuber size (12.36 ± 0.2a) and diameter of tubers (2.12 ± 0.05a) were also largest. In population- III, tuber size (7.6 ± 0.15c) and diameter of tubers (1.4 ± 0.04c) were smallest among the all populations but plants were larger than population- VI. Leaf length (10.0 ± 0.1a) of population-II was largest than other populations and leaf length (5.2 ± 0.4a) of population- V was smallest among the all populations but leaf width (4.5 ± 0.1a) and internodal length (8.76 ± 0.2a) was largest than other populations. Leaf width (2.0 ± 0.4b) of population- VI was smallest among the other five populations. In population I, III and V, leaf length and leaf width were nearly similar size. Internodal length (5.0 ± 0.15c) of population V was smallest among the all populations. Flower color of population V was also distinguishable and noticeable from other populations which were upper red and lower greenish in color (Fig. - F) and petals length was 4.8 ± 0.2b. Petals length (10.1 ± 0.1b) of population I was largest among the all populations and flower color was upper pinkish, lower white in color (Fig. - A).In population II, flower color was off yellow with arid patch along the central region (Fig. - B) and petals length (3.8 ±0.3c) was smallest then other populations (Table-2).

Table-2: Gross morphological analysis of different Morphotypes of *Gloriosa superba* L. Values represent means ± standard error of three experiments with 10 replicates.

Characters	I	II	III	IV	V	VI
Leaf length (cm.)	7.6 ± 0.2	10.0 ± 0.1	7.4 ± 0.3	8.8 ± 0.1	5.2 ± 0.4	6.6 ± 0.3
Leaf width (cm.)	4.4± 0.1	4.3 ± 0.2	4.4 ± 0.5	4.4 ± 0.1	4.5 ± 0.1	2.0 ± 0.4
Internodal length (cm.)	5.94 ± 0.13	6.32 ± 0.19	5.0 ± 0.15	5.08 ± 0.07	8.76 ± 0.2	5.88 ± 0.1
Tuber size (cm.)	10.21 ± 0.13	10.34 ± 0.14	7.7 ± 0.15	7.6 ± 0.16	8.82 ± 0.23	12.36 ± 0.2
Diameter of Tuber (cm.)	1.51 ± 0.02	1.62 ± 0.03	1.4 ± 0.04	1.34 ± 0.06	1.7 ± 0.05	2.12 ± 0.05
Flower color	Upper pinkish and lower white in color	Off yellow in color with arid patch along the central region	Lower light yellowish and upper brownish in color	Lower whitish-yellow and upper red in color	Lower creamy and upper red in color	Upper red and lower greenish in color
Length of petals	10.1 ± 0.1	3.8 ± 0.3	5.0 ± 0.2	4.9 ± 0.4	9.5 ± 0.2	4.8 ± 0.2

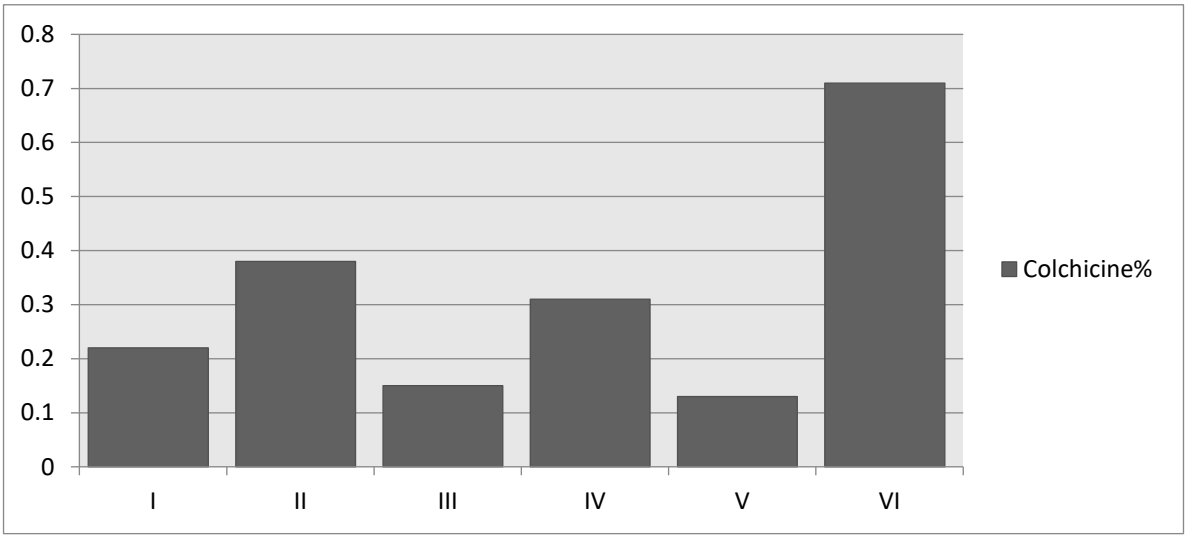
(Morphotypes details: I- Rahara, II- Narendrapur, III- Sargaachi, IV- Kalyani, V- Benapur, VI- Lava)

❖ **Variations in colchicine in different populations:**

Qualitative presence of colchicine in the tubers of the six collected populations necessitated the quantitative estimation of their colchicine content. Qualitative and quantitative estimation from the tuber extraction from the tuber of *Gloriosa superba* L. was done through HPTLC and HPLC. There were significant variations in colchicine content of all the six populations in West Bengal. The colchicine content in the tubers of *Gloriosa superba* L. ranged from 0.13 ± 0.01 to 0.71 ± 0.01. The height concentration of colchicine (0.71%) was observed in the population VI and the lowest concentration of colchicine (0.13%) was observed in the population IV. In our study, it was observed that variation of colchicine content depends upon the morphological variations of tubers (as on size and diameter) in different populations. In population VI, tuber size (12.36 ± 0.2a) and diameter of tuber (2.12 ± 0.05a) was larger among all the populations and colchicine content was also higher percentage than other populations. But in case of population-IV, tuber size (7.6 ± 0.1c) and diameter of tuber (1.34 ± 0.06a) was smallest among all the populations and colchicine content was also lower percentage than other populations. The variations of colchicine content in different populations are summarized in Table-2. Relatively high colchicine content of the population-VI encourages its cultivation in Lava of District of Darjeeling (Table-3).

Table-3: Colchicine content of tubers (1st yr. old) of the plants of the six morphotypes collected. Values represent means ± standard error of three experiments with 10 replicates.

Morphotypes	Colchicine content (% d.wt)
Morphotype- I	0.22 ± 0.02
Morphotype- II	0.38 ± 0.0
Morphotype- III	0.15 ± 0.01
Morphotype- IV	0.13 ± 0.01
Morphotype- V	0.31 ± 0.02
Morphotype- VI	0.71 ± 0.01



❖ **Cytological variations in different populations:**

Cytological analysis of the root tips of the plants revealed that among the six populations examined in this study and all were diploid in nature. Frequency of metaphase plates showing <22 chromosomes varied from 0.05- 0.09% and those showing >22 varied from 0 (Table-4).

Table-4: Comparative karyotype of different Morphotypes of *Gloriosa superba* L.:

Morphotypes	Source	No. of 2n chromosome	2n karyotype formula	Total chromatin length (µm)	Length of Longest chromosome (µm)	Length of Smallest chromosome (µm)	L-S (µm)	TF %	% of m	% of Sm	Total arm ratio	Range of length of chromosome (µm)
I	In vivo	22	2A + 2B + 8C + 10D	62.5± 0.12	10±0 .13	4±0. 44	6	38. 18	36. 4	45.5	2.46	4- 10
II	In vivo	22	2B + 14C + 6D	68±0. 79	11.5 ±0.42	5±0. 38	6. 5	39. 86	63. 6	27.3	1.94	5- 11.5
III	In vivo	22	2B + 14C + 6D	54±0. 02	10±0 .06	3.5± 0.41	6. 5	41. 60	63. 6	27.3	1.75	3.5- 10
IV	In vivo	22	2A + 16C + 4D	54±0. 05	10±0 .03	4±0. 05	6	39. 33	72. 7	18.2	1.64	4- 10
V	In vivo	22	2A + 16C + 4D	65.5± 0.42	11.5 ±0.30	3.5± 0.35	8	40. 05	72. 7	18.2	1.61	3.5- 11.5
VI	In vivo	22	2A + 2B + 12C + 6D	69.5± 0.88	10.5 ±0.20	4±0. 02	6. 5	39. 04	54. 5	27.3	2.47	4- 10.5
Morphotypes	Source	No. of 2n chromosome	2n karyotype formula	Total chromatin length (µm)	Length of Longest chromosome (µm)	Length of Smallest chromosome (µm)	L-S (µm)	TF %	% of m	% of Sm	Total arm ratio	Range of length of chromosome (µm)
I	In vivo	22	2A + 2B + 8C + 10D	62.5± 0.12	10±0 .13	4±0. 44	6	38. 18	36. 4	45.5	2.46	4- 10
II	In vivo	22	2B + 14C + 6D	68±0. 79	11.5 ±0.42	5±0. 38	6. 5	39. 86	63. 6	27.3	1.94	5- 11.5
III	In vivo	22	2B + 14C + 6D	54±0. 02	10±0 .06	3.5± 0.41	6. 5	41. 60	63. 6	27.3	1.75	3.5- 10
IV	In vivo	22	2A + 16C + 4D	54±0. 05	10±0 .03	4±0. 05	6	39. 33	72. 7	18.2	1.64	4- 10

V	In vivo	22	2A + 16C + 4D	65.5± 0.42	11.5 ±0.30	3.5± 0.35	8	40.05	72.7	18.2	1.61	3.5-11.5
VI	In vivo	22	2A + 2B + 12C + 6D	69.5± 0.88	10.5 ±0.20	4±0.02	6.5	39.04	54.5	27.3	2.47	4- 10.5

(Morphotypes details: I- Rahara, II- Narendrapur, III- Sargaachi, IV-Kalyani, V- Benapur, VI-Lava)

In columns 5, 6, 7 the results are mean ± Standard Error (calculated from 5 replicates)

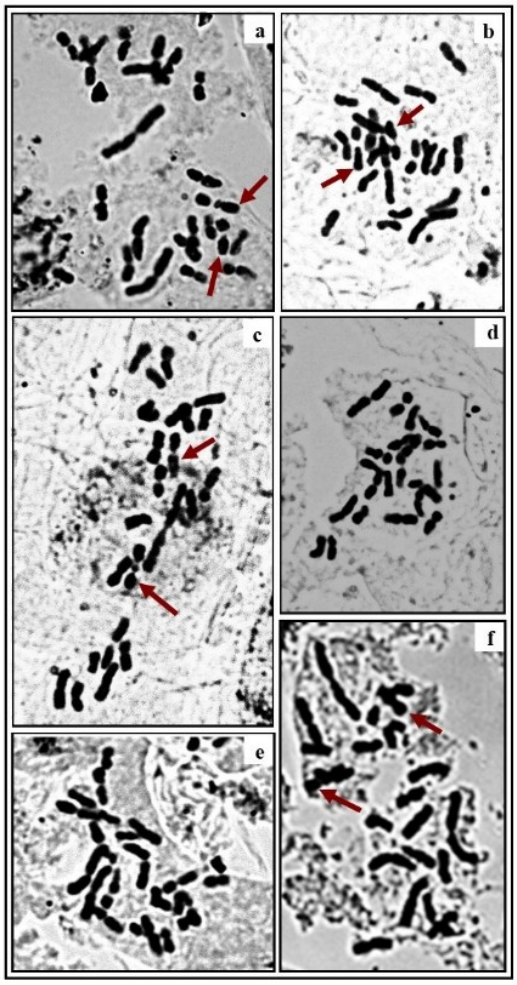


Fig. – (a- f); Showing 2n= 22 metaphase chromosome in different populations of *Gloriosa superba* L. from the state of West Bengal, a- Rahara , b- Narendrapur , c- Sargachi, d- Kalyani, e- Benapur, f- Lava. The arrows are representing the interstitial secondary constrictions.

5. Conclusion:

The variation of colchicine content, such as Table no.-3, may suggest there are no correlations between the karyotypes of the six morphotypes. The high colchicine content in the morphotypes of Lava may be due to the over expression of specific genes in these climatic conditions.

6. Acknowledgement

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