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Heterochromatin Distribution in the species of *Iphegenia* Kunth

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Abstract

Banding patterns were revealed in the somatic chromosome of 4 species Iphigenia Kunth using HCl-Giemsa staining techniques. There was considerable variation both regarding the amount and distribution of bands; C-banding of chromosome promises to be the most valuable technique for routine chromosome analysis due to its inherent simplicity, sensitivity and stability of the material obtained. HCl- Giemsa banding technique was used to study Heterochromatin (HC) banding pattern in genus Iphigenia. The genus Iphigenia is a monocot. The species studied here named as, I. pallida (Baker), I stellata (Blat), I. magnifica (A and R) and I. indica (Linn.) were characterized by 2n= 22 chromosome. The chromosome complement exhibited telomeric and centromeric HC. I. magnifica (A and R) and I. indica (Linn.) showed more banded chromosomes than I. pallida (Baker) and I. stellata (Blat). Telomeric bands were mostly present on short arm of the chromosomes.

Keywords: HCL-Giemsa banding, Heterochromatin (HC), Telomeric, Centromeric.

Introduction

The banding techniques have been useful in the identification of individual chromosome of the complement and in the analysis of chromosomal abnormalities both structural and numerical. These methods have also been used to understand the changes during evolution of the karyotype in a number of plant and animal species[1-6].

In recent years remarkable advances have been made in technique for the study of linear differentiation of chromosome. The techniques of chromosome banding have allowed the identification of heterochromatin segment of the individual chromosome. These heterocromatic segments are primarily composed of repetitive DNA[7] which is discernable in the form of dark staining region by different chromosome banding techniques depending upon the particular type of DNA repetition.

Several types of heterochromatin can be detected within the chromosomes heterochromatic segments following different techniques of banding such as Quinacrine fluorescence and Giemsa[8]. The number, position and density of bands produced on chromosome complement of a species may vary from technique to technique which reveals the presence of different class of heterochromatin within the same chromosome complement. Many research papers, after the development of HC differentiation technique in the metaphase chromosome of a great diversity of organism, reported its presence, variability, molecular composition, direct and indirect effects on karyotype, putative function, etc[9-12]. One reasearcher[13] compared C-band distribution patterns of 105 angiosperm species and identified general pattern for heterochromatin (HC).

Concentrated hydrochloric acid (HCl) treatment alone cause the conformational change in chromosome morphology. The occurrence of interstitial bands can be attributed to differential condensation of chromatin where as centromeric heterochromatin may be visualized because of a tight complex of DNA - non - histone protein[14].

In the present work, HCL-Giemsa banding techniques were applied to study the heterochromatin polymorphism in somatic chromosome of *Iphigenia* species having chromosome number 2n=22. In order to establish nature of heterochromatin in *Iphigenia* this technique was applied to chromosome from root meristem cells.

Material and methods

A protocol devised by Joshi and Ranjekar[15] was followed to resolve chromosomal heterochromatin. The root tips were pretreated with 0.1% colchicine at 4°C for 2-4 hrs. They were then fixed in acetic- alcohol (1:3) and stored in 70% ethanol. The root tips then washed and hydrolyzed in 1N Hydrocloric acid (HCl) for 5-7 minutes at room temperature and squashed in 45% acetic acid. Cover slip was removed either by mechanical method or by giving ethyl alcohol treatment. Prepared slides were dried. The air dried preparation was treated with 6N hydrochloric acid (HCl) for 10 - 20 mints.

After HCl treatment slides were thoroughly washed in cold running water, slide was stained with 4% Giemsa solution diluted with m/15 Sorensen phosphate buffer pH 6.8 (0.06M Na_2HPO_4 + 0.06M KH_2PO_4). The slide was made permanent by mounting in Canada balsam.

In the present investigation the position of the band was classified as telomeric when located in the terminal end of the chromosome, proximal when located in the proximal region or immediately after the primary constriction, and as interstitial or intercalary when they occupied neither of the chromosome arm extremities. When the band occupied the whole chromosome arm, it was classified as proximal or telomeric depending on the dominant band pattern in the Karyotype.

Results and discussion

Heterochromatin pattern(C- banding) studied in the species of *Iphigenia* (Table-1) showed that *I. pallida* (Baker) exhibited eight pairs (1, 3, 5, 6, 7, 8, 9, 10 and 11) of banded chromosomes. A single telomeric band was seen on chromosome pair number 3, 4, 8, 9, 10, 11. Out of these 8, 9, 10, 11 pair possessed dark telomeric band. Chromosome pair number 1 and 5 showed HC in the proximal region near by the centromere. While *I. stellata* (Blat) showed nine (1, 3, 5, 6, 7, 8, 9, 10 and 11). Chromosome pair no. 1,3,5,7 and 8 showed bands in the proximal region near the centromere while in chromosome pair no. 10 HC was in the form of a light telomeric band. While pair no 9 showed dark and thick telomeric band. The chromosome pairs 2 and 4 did not show any C-band. In *I. magnifica* (A and R) HC was present in ten pairs (1, 2, 3, 4, 5, 6, 7, 8, 10 and 11). Centromeric HC bands were seen in 7 chromosome pairs namely chromosome no. 1, 2, 3, 6, 7, 8 and 9. Out of these chromosome pairs 2 and 6 exhibited light centromeric band, where as chromosome pairs 4, 5 and 11 showed telomeric bands. However in *I. indica* (Linn.) all the chromosome pairs showed HC bands, of the 11 pairs, chromosome no. 3, 4, 5, 7, 8, 9 and 10 contained proximal (centromeric) HC band. Chromosome pair no. 7 showed light centromeric band than the other chromosomes. The chromosome pair no. 1, 2, 6 and 11 exhibited HC in telomeric region on the short arm. The chromosome pair no. 2 showed very light telomeric band. Among the species of *Iphigenia* frequency of chromosome with proximal HC was more than the telomeric HC. Only *I. pallida* (Baker) chromosome complement contained less proximal HC. Among the longer chromosome of the complement, which can be easily identified, those of *I. magnifica* (A and R) and *I. pallida* (Baker) show centromeric and telomeric bands. The longer chromosome of *I. stellata* (Blat) and *I. indica* (Linn.) can be characterized by presence of only proximal centromeric and telomeric HC band respectively. The chromosome of the complement in *I. pallida* (Baker) had two proximal while rest seven had telomeric HC. *I. stellata* (Blat) had a mixture of proximal and telomeric HC in more or less equal proportion. In the complement of *I. indica* (Linn.) and *I. magnifica* (A and R) occurrence of proximal HC was more common. A Giemsa stained metaphase of *Iphigeni*a is presented in Figure-1 and Idiogram in Figure-2.

Overall the number of proximal HC band is more than that of telomeric HC band in species of *Iphigenia.* This is in concurrence with the report from Vosa[16] that occurrence of any detectable kind of proximal HC is much higher and it may be a common feature of all angiosperms.

The most likely mechanism of C-banding is that the DNA of heterochromain (HC) is protected from extraction by non-histone proteins specific to this (HC)[17].

The variation of C-band among eleven pairs as well as difference in intensity and size of C-band were observed in four species of *Iphigenia*. Such variation referred to be as band polymorphism or band heteromorphism[18]. C-band heteromorphism has been reported in plants by Researchers[19-22]. The presence of heterochromatin increases the frequency of interchanges[23], which may be responsible for shifting the position of band from one to another chromosome.

As revealed by literature survey[24-26], the analysis of c-band patterns in more than one hundred angiosperm, Karyotype with different chromosome sizes showed that HC is preferentially located and not randomly distributed on chromosome. This HC type is positively stained by C- banding techniques very frequantly, and GC- rich chromatin show high affinity for fluorochromes. The remaining HC is mainly AT- rich and, regardless of its DNA base composition, it has a generalized equailocal distribution. It is commonly observed that HC band in small chromosomes is distributed at proximal region. It is true in *Iphigenia* also. High frequency of prochromosomal nuclei is related to the proximal bands in species with small chromosomes.

Table-1: Distribution of Heterochromatin in *Iphigenia* on chromosomes

Name of Species	Chromosome pair Number exhibited HC (Heterochromatin)	HC absent in Chromosome pair	Chromosome pair exhibiting telomeric band	Chromosome pair exhibiting proximal band (near centromere)
I. pallida(Baker)	1, 3, 4, 5, 6, 7, 8, 9, 10 and 11	2	3, 4, 8, 9, 10, 11	1 and 5
I. stellata (Blat)	1, 3, 5, 6, 7, 8, 9, 10 and 11	2, 4	1, 3, 5, 7 and 8	9 and 10
I. magnifica (A and R)	1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11	-	4, 5 and 11	1, 2, 3, 6, 7, 8 and 9.
I. indica (Linn.)	1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11	-	1, 2, 6 and 11	3, 4, 5, 7, 8, 9 and 10

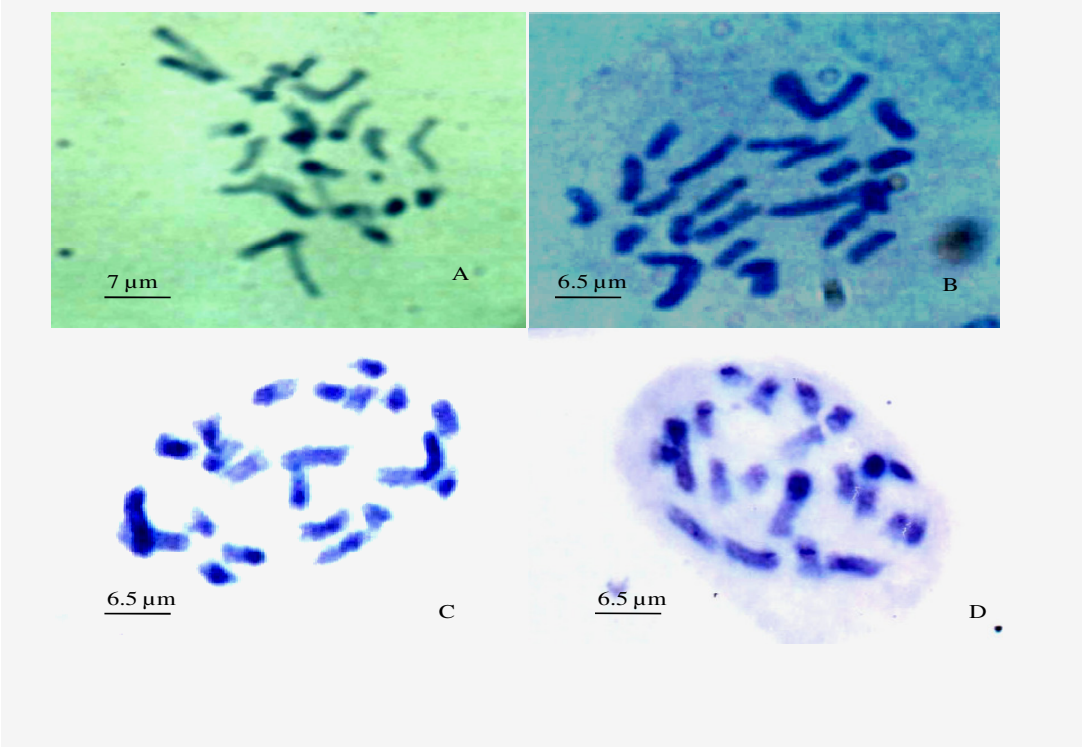

Figure-1: Heterochromatin pattern (C-banding) in *Iphigenia* A- *I. pallida*, Baker. B- *I. stellate*, Blat. C- *I. magnifica,* A. and R. D- *I. indica* L.

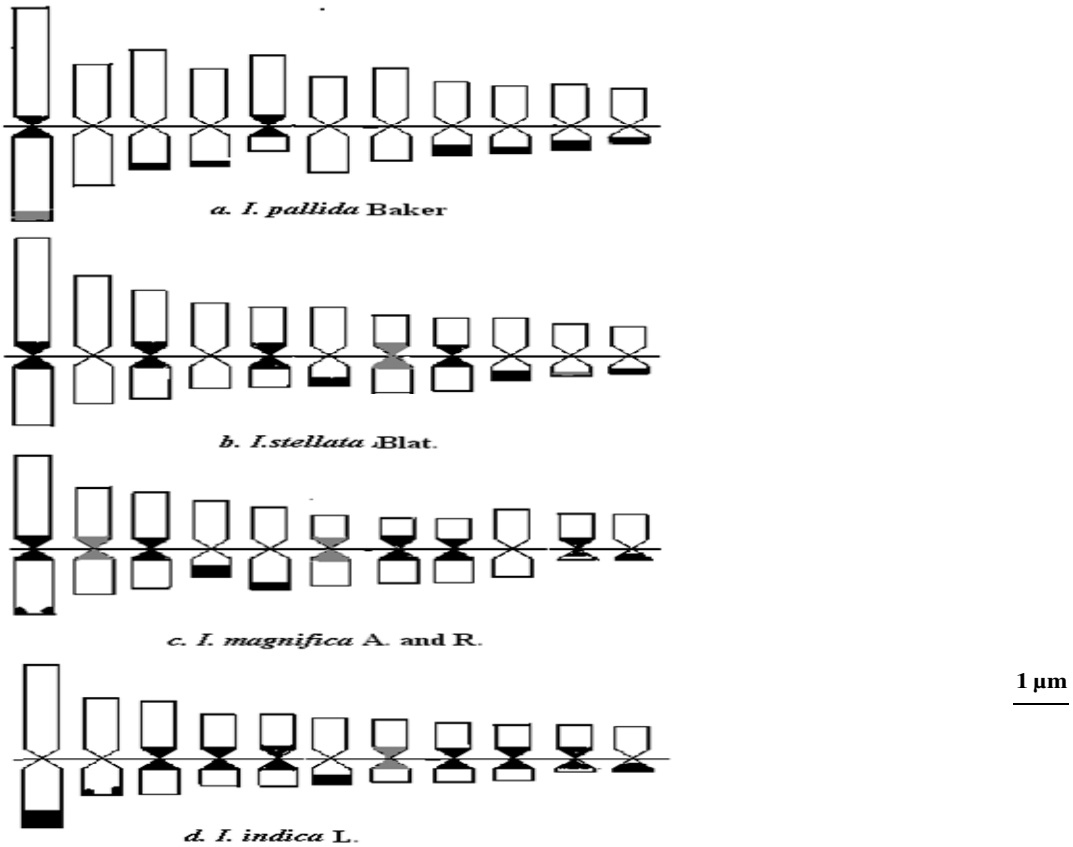

Figure-2: Ideograms of *Iphigenia* species. a**.** *I. pallida*(Baker), b**.** *I. stellata*(Blat.), **c** *I. magnifica*(A & R), d *I. indica*(Linn.)

Conclusion

The chromosome complement exhibited telomeric and centromeric HC. *I. magnifica* (A and R) and *I. indica* (Linn.) showed more banded chromosomes than *I. pallida* (Baker) and *I. stellata* (Blat). Telomeric bands were mostly present on short arm of the chromosomes. It is commonly observed that HC band in small chromosomes is distributed at proximal region. It is true in *Iphigenia* also. High frequency of prochromosomal nuclei is related to the proximal bands in species with small chromosomes.

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