

**Influence on Chromosome Behaviour, Nucleic Acid Content  
and Ultra-Structural Analysis of Accessories  
in *Urginea indica* KUNTH.**

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**Abstract.** The occurrence of polysomaty was noted in a diploid cytotype of *Urginea indica* with B chromosomes. These accessories could be clearly distinguished from the other chromosomes in their stainability, very small size, morphology, variability and irregular behaviour. The variant nuclei were hyperdiploid, hypertriploid and hypertetraploid. B chromosomes were noted only in diploid cells and no accessories could be recorded in cells showing higher chromosome number.

The amount of DNA content as indicated by the absorbance is rather low as compared to the diploid without accessories which may be due to the shorter size of the chromosomes. Under ultrastructural study subterminal centromeric region of B chromosome show low electron dense appearance.

In *Urginea indica*, SEN (1974) recorded the presence of B chromosomes in diploid populations. No such accessories were recorded in polyploids. SHARMA and AIYANGAR (1961) reported the presence of B chromosomes only in diploid *Allium stracheyii* and polyploids were found to be completely devoid of such accessories. DARLINGTON (1956) recorded that B chromosomes are more frequent in diploids than in polyploids.

In connection with the investigation of Indian squill, the occurrence of polysomaty was noted in a cytotype with B chromosomes. An analysis of the structure and behaviour of such a cytotype has been dealt with. Moreover, data on the effect of B chromosomes on DNA content and the ultrastructural pattern of the B chromosomes are presented.

**MATERIAL AND METHODS**

The bulbs were collected from Bangalore and grown in the experimental garden. Root tips were cytologically analysed as reported earlier (JHA and SEN 1983). Root tips were pretreated with 0.05% colchicine for 2—3 h to get C-metaphases. For Feulgen staining fixed root tips were hydrolyzed in normal HCl at 56—58 °C for 10 min and then stained with Schiff's reagent followed by squashing in 45% acetic acid. In root tip showing polysomaty, 150 metaphases were analyzed.

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*In situ* quantitative measurement of DNA was made through single wavelength method from 4C nuclei at metaphase in root tips following standard feulgen staining technique (McLEISH and SUNDERLAND 1961). For each cytotype, measurements from at least 50 nuclei (4C) were taken in a Reichert Zetopan with microphotometer and the average DNA with SE were calculated in arbitrary units.

For ultrastructural study the root tips were fixed in gluteraldehyde and also in osmic acid in phosphate buffer at pH 7.0. Standard araldite resin was used for embedding. The ultra-thin sections were stained with uranyl acetate and lead citrate and observed under a Corinth 500 microscope. The photographs were taken in 70 mm Ilford film.

## RESULTS

### Structure of B Chromosomes

After staining with Feulgen solution, the B chromosomes were found to be deeply stained in both early and late prophase (Fig. 1). The general tendency noted amongst these accessories was to remain mostly in groups of two (Fig. 2). The small size and subterminal constriction are rather permanent features. The number of B chromosomes was found to vary from cell to cell, the most frequent number being six (Fig. 3).

In this population, one root tip was found to show polysomaty. Nuclei were recorded with a varying number of chromosomes. Out of these 18% of cells were hyperdiploid, 15% were hypertriploid and 8% were hypertetraploid. It is remarkable that cells with a high number do not have any B chromosomes (Fig. 5). That the variation in number is not due to B chromosomes is indicated by their morphology, size, staining and regular behaviour on the spindle.

TABLE 1  
Average DNA value in arbitrary units (4C nuclei)

| Material | DNA value $\pm$ S.E. |
|----------|----------------------|
| 2n       | 16.84 $\pm$ 0.84     |
| 2n + B   | 11.56 $\pm$ 0.65     |
| 4n       | 25.10 $\pm$ 0.88     |

### DNA Content

The 4C DNA content of the diploid cytotype with and without B chromosomes and of a tetraploid cytotype without B chromosomes have been determined (Table 1). The amount of DNA as indicated by the absorbance is rather low in the population with B chromosomes as compared to that without them. The tetraploids do not show exact multiples of the diploid set.

### Ultrastructure of B-Chromosomes

As far as the ultrastructural pattern of B chromosomes is concerned, their small size and comparatively lesser electron density in the proximal region

are distinct (Fig. 6). The subterminal nature of the constriction has been seen in light microscope preparations. Evidently, the low electron dense portion may be associated with centromeric segment.

### DISCUSSION

A conspicuous feature in the above observation is the presence of B chromosomes only in the diploid cells. In none of the cells showing a high chromosome number could such accessories be recorded. The origin of polysomaty may be due to non-disjunction, the hypodiploid number possibly not surviving. No hypodiploid cell was observed.

Previous record (SEN 1974) as well as the observation presented in this paper show the occurrence of B chromosomes only in diploid population ( $2n = 20$ ) and their complete absence in cytotypes with a higher chromosome number. In the present observation, even in the individual with B chromosomes, cells having a higher chromosome number have been completely devoid of accessories even though occurring in the same root tip, forming a polysomatic tissue.

Regarding the DNA content, its low nature in individuals with accessories is of special interest. However, in populations with B chromosomes, no individual could be located without such an accessory. The diploid without the accessories evidently belong to a different population where chromosome size, too, has been found to be rather long (length =  $94 \mu\text{m}$ ). All individuals of the populations are thus characterized by an overall increase in chromosome size (JHA and SEN 1983). The low DNA value in individuals with accessories is attributed to the shorter size of the chromosomes (length =  $58 \mu\text{m}$ ) as compared to the that of individuals without B chromosomes. The variation in DNA content between different populations is thus of special interest. The extent to which such variation is associated with increase or decrease of amplified sequences is yet to be established. The present data provide further evidence of the effect of B chromosomes on the overall DNA content of the genome. Incidentally, the tetraploid population also does not show the exact multiple of that of the diploids so far as DNA content is concerned. This may also be due to genotypic control and the diploid genotype of the same might have been different from the diploids studied.

In relation to the chromosome ultrastructure, the accessories do not reveal significant differences from the rest of the chromosomes of the genome. Only the centromeric region shows a low electron dense appearance not recorded so prominently in the rest of the chromosomes.

### REFERENCES

- DARLINGTON, C. D.: Chromosome Botany and the Origin of Cultivated Plants. 3rd Ed. — G. Allen & Unwin Ltd., London 1956.
- JHA, S., SEN, S.: Chromosome study of diploid Indian squill. — *Cytologia* **48** : 79–86, 1983.
- MCLEISH, J., SUNDERLAND, N.: Measurements of DNA in higher plants by Feulgen photometry and chemical methods. — *Exp. Cell Res.* **24** : 527–540, 1961.
- SEN, S.: Nature and behaviour of B-chromosome in *Allium stracheyii* BAKER and *Urginea indica* KUNTH. — *Cytologia* **39** : 245–251, 1974.
- SHARMA, A. K., AIYANGAR, M. R.: Occurrence of B-chromosomes in diploid *Allium stracheyii* BAKER and their elimination. — *Chromosoma* **12** : 310–317, 1961.