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GIEMSA C-BANDING, SOMATIC ASSOCIATION AND ORIENTATION OF INTERPHASE CHROMOSOMES IN *TRIGONELLA FOENUM-GRAECUM* (L.)

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INTRODUCTION

Interphase chromosomes are generally described as very long uncoiled strands of nucleoprotein. Such chromosome filaments are not freely dispersed in the nucleoplasm. They are attached at several points to the nuclear membrane (MAZIA 1961; DUPRAW 1965*a*; COMINGS and OKADA 1970), possibly at the level of nuclear pores (DUPRAW 1965*b*; COMINGS 1968) and have definite arrangement in the interphase nucleus. The nonrandom distribution of interphase chromosomes has been demonstrated by several authors (HEITZ 1932; VANDERLYN 1948; WAGENAAR 1969; STACK and CLARK 1974; FUSSEL 1975; CHURCH and MOENS 1976; GHOSH and ROY 1977; ROY and GHOSH 1977). Further, it has also been shown that the relative position of homologous chromosomes during mitosis tends to be nonrandom (SCHNEIDERMAN and SMITH 1962; KITANI 1963; FELDMAN *et al.* 1966; HENEEN and NICHOLS 1972; FELDMAN and AVIVI 1973; JURICEK 1975; ARDITO 1976; SINGH *et al.* 1976; FERRER and LACADENA 1977).

Giemsa C-banding technique which preferentially delineates the constitutive heterochromatic regions on the chromosomes offers a means of studying orientation and arrangement of interphase chromosomes and other stages during mitotic movements. The present study deals with the chromosome banding patterns, somatic association and orientation of chromosomes during various stages of mitotic cell cycle as detected by Giemsa C-bands in *Trigonella foenum-graecum* (L.).

MATERIALS AND METHODS

a) *Giemsa C-banding*. Root tips from fast growing roots of *Trigonella foenum-graecum* were pretreated for three hours in saturated aqueous solution of para-dichlorobenzene at 14-16°C and fixed overnight in acetic-ethanol (1:3). The fixed root tips were hydrolysed in N. HCl for 40 seconds at 60°C and squashed in 45% acetic acid. The coverglass was detached by inverting the slide in absolute ethanol and preparations made air dry. For C-banding, air dried preparations were treated with saturated aqueous solution of barium hydroxide for 5 minutes at 20°C, rinsed in water and incubated in 2X SSC at 60°C for one hour, washed in distilled water and stained in 5% Giemsa in M/15 phosphate buffer pH 6.8 (VOSA and MARCHI 1972). The scattered metaphase plates were studied for C-banding patterns.

b) *Somatic association and orientation of Giemsa C-bands*. In another set, root tips were directly fixed in fixative and processed as above for C-banding. Observations were made on prometaphase and certain metaphase plates where chromosomes could be identified. Besides, observations were recorded on the orientation and arrangement of chromosomes and Giemsa C-bands during various stages of mitotic cell cycle. Approximately 25 cells each from 10 replications were recorded for the analysis.

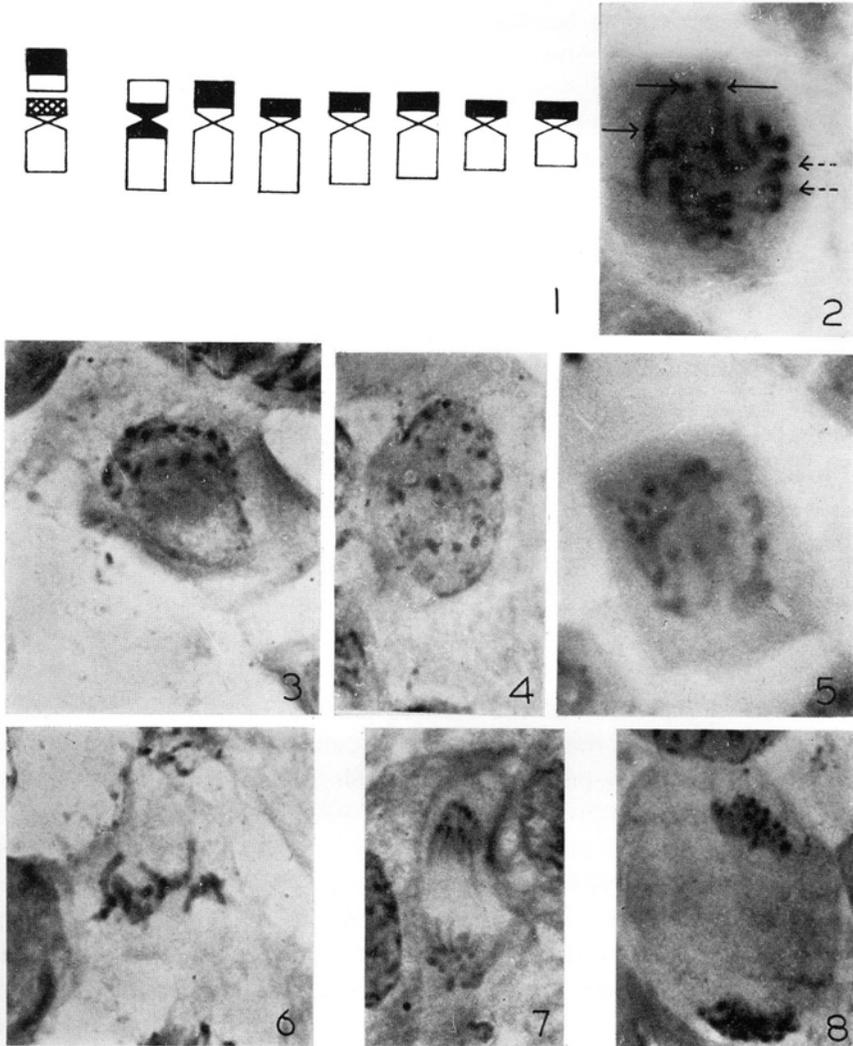
OBSERVATIONS

Giemsa C-banding. Somatic metaphase chromosomes of *Trigonella foenum-graecum* show one block of Giemsa C-band at the telomeric end of each of the 8 chromosomes on the short arm except chromosome No 2 where it is in the centromeric region. The nucleolar chromosomes (only one pair present in this cytotype) have bands at the terminal end of the distal arm and in the region of secondary constriction (Fig. 1). Total number of Giemsa C-bands on the chromosomes generally corresponds with the number of Giemsa positive dots (14-18) at interphase.

Somatic association. From the observation of prometaphase plates (Fig. 2) it appears that homologous chromosomes lie close to each other. Though chromosome morphology is not very clear at this stage, yet some of the homologous pairs can be easily identified with the help of Giemsa C-bands and size of the chromosomes. In some of the proper metaphase plates (obtained without any pretreatment), where the chromosomes could be identified to some extent, the two nucleolar chromosomes were observed lying adjacent to each other.

Orientation of interphase chromosomes.

In interphase nucleus Giemsa C-bands form a ring like arrangement towards one pole (Fig. 3). When the nucleus is viewed from polar view



- Fig. 1. — Giemsa C-banded karyotype ($n=8$).
 Fig. 2. — C-banded prometaphase.
 Fig. 3. — Interphase cell showing heterochromatic ring on one pole.
 Fig. 4. — Interphase cell in polar view after nuclear rotation. Heterochromatic ring and a few Giemsa positive dots in the central gap of the ring are clearly visible.
 Fig. 5. — Prophase with C-bands.
 Fig. 6. — Metaphase chromosomes (C-banded) arranged at equatorial plate.
 Fig. 7. — Anaphase with C-bands. Note, vessel like arrangement of chromatids at two poles. On the poleward side heterochromatic ring is clearly visible.
 Fig. 8. — Telophase with C-bands.

the heterochromatic ring becomes clearly visible. A few Giemsa positive dots are present in the central gap (Fig. 4) representing possibly nucleolar segments.

When the interphase cells enter into prophase, chromosome arms become apparent (Fig. 5). Towards metaphase the chromosomes get arranged at the equatorial plate. Giemsa C-band regions of the chromosomes lie on the equatorial plate, perhaps because of the close proximity to centromeres (Fig. 6). (In general the chromosomes are acrocentric and the Giemsa bands are present on short arms). At anaphase, the sister chromatids of the chromosomes separate and move to the opposite poles. Here, all the chromatids during their poleward movement form a hollow vessel-like structure. A heterochromatic ring on the polar side can be clearly seen (Fig. 7). The same configuration is maintained at telophase too (Fig. 8) followed by interphase of the daughter cell (Fig. 3). Thus anaphase orientation of chromosomes is maintained till next interphase through telophase and chromosomes remain polarized during these stages.

DISCUSSION

Somatic association. As the observations reveal, the banding technique may be used in the study of the relative position of the chromosomes and their association among themselves during somatic division. The nonrandom distribution of chromosomes in somatic cells of plants has been reported by a number of authors (SADASIVAIAH *et al.* 1969 and THOMAS 1973 in oat; STACK and BROWN 1969 in *Plantago*; FEDAK and HELGASON 1970 in barley; FELDMAN and AVIVI 1973; MELLO-SAMPAYO 1973 in wheat; SINGH *et al.* 1976 in rye; FERRER and LACADENA 1977 in *Crepis* spp.). Various authors have applied various ways to find out the arrangement and relative positions of chromosomes in the cell nucleus. With the aid of heterochromatic knobs, MAGUIRE (1967) observed homologous association of somatic and premeiotic interphase chromosomes in maize. Non random arrangement of chromosomes was also deduced by RAO and NATARAJAN (1967) from a preferential formation of chromatid aberrations between homologous chromosomes after irradiation or chemical treatment in *Vicia faba*. From the cytological studies on telophase and early prophase in root tip cells of several plant species WAGENAAR (1969) concluded that chromosomes are attached end to end and form chain like interphase associations. In these interphase associations the chromosomes may be arranged in a specific order and homologous chromosomes are presumably located adjacent to each other. Somatic association of homologous rye chromosomes was determined in the wheat-rye addition lines (SINGH *et al.* 1976) by observing the Giemsa stained chromocenters in interphase

nuclei. FERRER and LACADENA (1977) analysed the chromosome arrangement in radial metaphases from root tips in *Crepis* spp. and occurrence of somatic association of homologous chromosomes was clearly demonstrated. LACADENA and PUERTAS (1969); FERRER and LACADENA (1977) suggested that the functional genetic identity of homologous chromosomes has its cytological expression. The adjacent location of homologous chromosomes in the interphase association would facilitate rapid and regular synapsis (WAGENAAR 1969). The present observations too indicate somatic association of homologous chromosomes in *Trigonella foenum-graecum*, which can be detected through Giemsa banding technique. The non-random arrangement of chromosomes throughout the division cycle is also indicated.

Orientation of chromosomes at interphase and other stages during mitotic movements.

The constitutive heterochromatin is present in the telomeric regions on the short arm of the chromosomes and a heterochromatic ring is clearly visible. This ring like configuration of C-band regions during the division stages is possibly the reflection of centromeric ring because of the close proximity of these heterochromatic blocks to the centromere. The ring like configuration of heterochromatic regions during anaphase movements is maintained in the interphase nucleus through telophase. When this interphase nucleus (Fig. 3) is viewed from polar view (Fig. 4) possibly after nuclear rotation (STACK and CLARK 1974) the chromocenters become clearly visible forming a ring. In the central gap of the ring a few heterochromatic masses could also be seen representing C-bands of chromosomes with nucleolar organizing regions. That the nucleolar interstitial C-bands remain in the nucleoplasm has been reported by GHOSH and ROY (1977) in *Allium cepa* from their observations of C-bands and electron micrographs of interphase nucleus. Interphase chromosomes may possibly remain attached to the nuclear membrane through heterochromatic regions. The position occupied by prometaphase chromosomes (Fig. 2) in fact represents the interphase position as suggested by FELDMAN and AVIVI (1973). Here all the heterochromatic regions on the chromosomes are oriented towards periphery.

The position of heterochromatic C-bands in interphase (Fig. 3) clearly indicates that chromosomes remain polarized in the nucleus and this polarization is a relic of anaphase-telophase chromosome orientation from the preceding division. The mechanisms involved in maintaining this chromosome orientation are not fully known. One possibility is that heterochromatic regions rich in repetitive DNA sequences may serve as points of attachment to the nuclear membrane (VANDERLYN 1949) which

may well provide a mechanism for holding chromosomes in place within the nucleus (FUSSEL 1975). The evidence that telophase orientation of chromosomes is maintained in a wide variety of plant species has been reported by various workers (VANDERLYN 1948; STACK and CLARK 1974; FUSSEL 1975; CHURCH and MOENS 1976; GHOSH and ROY 1977 and ROY and GHOSH 1977). Interpretations of the effects of radiation on chromosome breakage have also led to the suggestion that chromosomes are polarized within the cell (KITANI 1963; FOX 1966 and JANCY 1975).

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SUMMARY

Giemsa C- bands on somatic chromosomes of *Trigonella foenum-graecum* are present at telomeric end of short arm of chromosomes except chromosome number 2 where the band is centromeric and the nucleolar chromosomes where the bands are present on terminal end of distal arm and in the region of secondary constriction.

Arrangement of somatic chromosomes in mitotic cells is non-random and nucleolar chromosomes lie closely associated. At interphase, chromosomes remain polarized and heterochromatic regions form a heterochromatic ring towards one pole. The ring like configuration of heterochromatic regions at anaphase is maintained till next interphase and even early prophase through telophase. The regularity of non random arrangement of chromosomes throughout the division cycle is indicated.