Nigerian Journal of Botany, Volume 3, 191 – 195, 1990.

CHROMOSOMAL INTERCONNECTIONS AND METAPHASE I CLUMPING IN MEIOSIS OF FOUR SPECIES OF *CROTALARIA* LINN

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(Received December 23, 1988; Revised November 7, 1989)

ABSTRACT

The occurrence of interchromosomal connections in four species of *Crotalaria*: - *C. retusa, C. goreensis, C. cylindrocarpa* and *C. doniana* is reprorted. Clumping of metaphase I chromosomes is also reported. The degree of incidence of the interchromosomal connections and metaphase I chromosomes clumping have been determined. The proleable relationship oetween the chromosomal connections and metaphase I clumping, and the possibility of their being a useful ecological adaptation are discussed. The chromosome numbers of the four species are reported.

INTRODUCTION

Crotalaria retusa Linn, *C. goreensis* Cruill & Per,. *C. cylindrocarpa* D. C. and *C. doniana* Bak are common species of the genus *Crotalaria* L. in the Southern part of Nigeria. Their growth and vegetative habits are generally stable or uniform in various ecological locations, with slight variations in size under different soil conditions.

The occurrence of interconnections between non-homologous chromosomes in some stages of mitosis and meiosis in a number of plants and animals has been reported by a number of workers (Callen and Lloyd, 1960; Hoskins, 1968; Du Praw, 1970; Myhra and Brogger 1975; Viinikka and Nokkala, 1981). Chromosomes during cell division usually occur as seperate entities but sometimes there occur chromatin threads connecting the individual chromosomes. Often these interconnections are so thin that they cannot be picked with the light microscope (Emmerich et al, 1973). Golomb and Bahr (1974) reported the occurrence of interconnections in human chromosomes using the scanning electron microscope, and Myhra and Brogger (1975) observed that the interconnections contain DNA. But generally the occurrence or existence of these interconnections has been overlooked, thus their functions are not understood.

This report is the first of the occurrence of interconnection in the genus *Crotalaria* and the first report of its probable relationship with chromosome clumping.

MATERIALS AND METHODS

Young flower buds from the four species of *crotalaria* were collected from the wild between 8.00 a.m. and 9.00 a.m. and fixed directly in 1:3 (V/V) acetic acid – ethanol, and stored in the refrigerator for at least 24 hours before examination.

Meiotic studies were made from young anthers squashed in FLP orcein stain on freshly washed slides. The slides were allowed to stand for 10 hours with regular irrigation to avoid dehydration and then examined under the microscope. Photomicrographs were taken from good preparations.

Records of number of cells with interconnections and the number of bivalents involved per cell at diplonema and diakinesis were also kept.

RESULTS

Table 1 shows the percentage frequency of cells with elements showing interconnections and clumping. Interconnections were more visible at diplonema and diakinesis, and clumping were more evident at metaphase I in these four species. The chromosome numbers of the four species are also presented in Table 1.

TABLE 1

Frequencies of interchromosomal connections in diplotene and diakinesis and clumped metaphase I and chromosome numbers in four species of Crotalaria.

Species Name	*Number of Chromosomes*	*No. of cells*	*Diplotene % of Cells with bivalents linked* 2	3	4	*No of Cells*	*Diakinesis % of Cells with bivalents linked* 2	3	4	*No. of Cells*	*Metaphase % of cells with bivalents* 2	3	4	*Clumping*
Crotalaria retusa	2n = 16	105	42.9	29.5	20.9	95	31.6	25.3	18.9	102	10.8	7.8	4.9	30.4
Crotalaria goreensis	2n = 16	115	15.5	14.5	14.5	97	12.4	9.3	5.3	99	9.1	6.1	4.0	27.5
Crotalaria cylindrocarpa	2n = 16	112	11.6	16.1	15.2	86	12.8	10.5	8.1	92	9.8	5.4	5.4	26.5
Crotalaria doniana	2n = 16 + 2*	98	12.2	3.2	11.2	74	10.8	6.8	6.8	89	7.9	3.4	1.1	14.7

**The value here represents accessory chromosomes.*

At the premeiotic stage the chromosomes form a tightly bundled mass within the nucleus, and their appearance as paired structures is not readily discernible. Later, evidence of pairing and interconnections become clear (Plates I A, B. & C).

At diplotene stage (Plates I A & B) many interconnections between the heterochromatic regions could be seen. This was most intense in *C. retusa* and least so in *C. doniana.* There were fewer interconnections in diakinesis than at diplotene stage. The interconnections were usually terminal and between the three largest bivalents.

Clumping at metaphase I and some diakinesis cells, was also a significant observation (Plates I C & D). Clumping at metaphase I was most prevalent in *C. retusa* and least so in *C. doniana.* (Table 1).

In later stages of division, the interconnections were less evident. Thus there was no evidence of these events during the second meiotic division except some indication of their possible persistence in metaphase II (Plate I E).

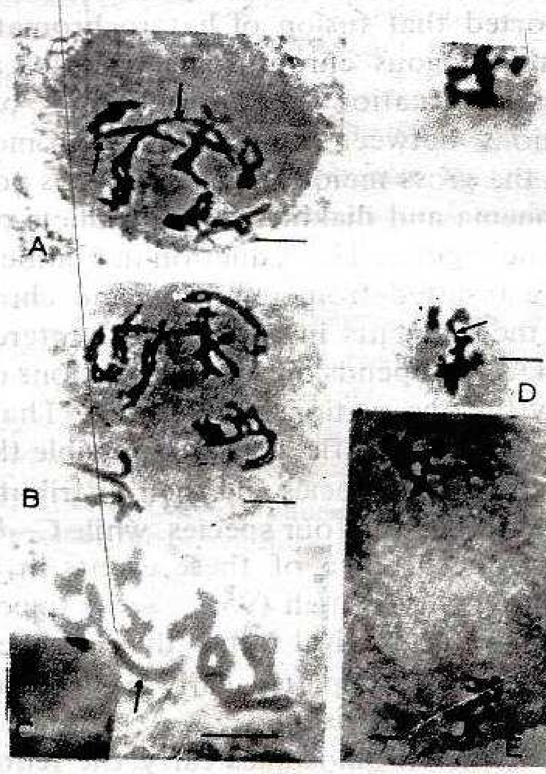

PLATE I A & B: Chromosome interconnections at diplotene in *C. retusa;*

C: Stretched interconnections at diakinesis in *C. goreensis;*

D: Metaphase I in *C. doniana* showing clumped elements and interchromatin connections;

E: Metaphase II in *C. cylindrocarpa* showing persistent interchromosome connections.

Arrows indicate the chromatin connections.

Bars represent 1 um.

The high percentage frequency of occurrence of the clumpings had no adverse effect on the percentage seed set and viability of seeds which were also very high.

DISCUSSION

Meiotic studies in a number of *Crotalaria* species have been done (Gupta and Gupta, 1975; 1976; 1977; 1978; Akpabio, 1986). Akpabio, (1986) observed that the meiotic elements of *Crotalaria* species have a high tendency to cluster together as evident at metaphase I (Plate I). The observations reported in the present paper could suggest that there is a direct relationship between chromosome interconnections and clumping or clustering of these elements.

During the first meiotic division in the *Crotalaria* species studied, the heterochromatic regions of some chromosomes were seen to fuse together. This probably results in the formation of chromatic knots as coiling occurs.

However, it has been reported that fusion of heterochromatic parts is not important in the pairing of homologous chromosomes (Miklos and Nankivell, 1976; Yamamoto, 1979). A similar situation was reported in rye by Viinikka and Nokkala (1981). Interconnections between homologous chromosomes would make little or no difference to the gross meiotic processes. It is possible that the prominent connections in diplonema and diakinesis are products of the preceeding fusion of the heterochromatic regions. The reduction in number and intensity as meiosis progresses must have resulted from repulsion and chromatin breaks.

From the consistency of the elements involved in the interconnections, it shows that the affiliation is genic and depends on specific portions of the chromosomes. This view is supported by the observations in Plate I A. That is, the portion involved in the interconnections is very specific. It is also possible that the intensity of occurrence is a characteristic of the species and their distribution. *Crotalaria retusa* has the widest distribution out of the four species, while *C. doniana* has the narrowest distribution. The non—occurrence of these events in later stages of division and the fact that pollen fertility is high (95%), seem to complement the view that they are a useful strategy for survival rather than an aberration. Apparently the genetic exchange process is completed early in division and normal division processes characterise the second division.

It is also possible that the large chromosomes carry the required genes for adaptation to various ecological environments and thus their consistent involvement in interconnections. This will not preclude that the smaller chromosomes did not have similar factors which were resolved in the present study. On the other hand, it can be argued that the phenomenon of chromosome interconnections is to ensure a regular behaviour of these large bivalents during metaphase I condensation and anaphase I movement.

Chromosome clumping in plant and animal cells is a very usual observation. A number of explanations have been advanced for the cause as well as nature of chromosome clumping. Chromosome clumping has always been limited to dead cells and degeneration. Generally the occurrence of chromosome interconnections and its probable relationship with clumping of chromosomes have been overlooked and thus very little is understood about these two observations. From this study it appears that clumping and chromosome interconnections are related and are a mechanism for survival and ecological adaptation for the different species. Further investigations are however needed to confirm these viewpoints.

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