CYTOPLASMIC CLEAVAGE DURING MICROSPOROGENESIS IN *AGROPYRON CRISTATUM*

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Abstract: *Agropyron cristatum* ($2n=14$), CB-9-85, was obtained from seeds treated with 0.1% colchicine. The spikes of F_1 progeny of CB-9-85 from outcross were collected for cytological observation. Furrowing process occurring during microsporogenesis was found. Supernumerary cytoplasmic cleavage of meiocytes, supernumerary microcells, and micronuclei occurring in quartet were also observed.

INTRODUCTION

Cytoplasmic cleavage has been reported occuring in both plants and animals (Tucker, 1971; Cutter and Hung, 1972). The two broad mechanisms of cell division are: 1) the development of the phragmoplast which principally found among meristematic tissue of high plants, and 2) the formation of cleavage furrows which is associated with the cells of animals. However, in microsporogenesis of pollen mother cell among plants may involve both above processes (Esau, 1965).

Karyokinesis (nuclear division) and cytokinesis appear to follow each other so closely that karyokinesis and cytokinesis appears to be one phenomenon; however, the two processes may be separate. In certain animal cells, which have entered metaphase, cleavage can occur even if the spindle is removed or destroyed (Mazia, 1961). Injured human amnion cells in which cytokinesis has begun, application of *p*-DL-fluorophenyl alanine will not stop cleavage, but chromosome movement does slow down in anaphase (Sisken, 1973). In plant cells, cytokinesis can be inhibited by certain concentrations of caffeine (Pickett-Heaps, 1969) and deoxyguanine (Brulfert, *et al.*, 1974) without interrupting nuclear division. In potato meiocytes, the cytoplasm can undergo cleavage and form a quartet, triad or dyad, irrespective of the division or the restitution of the nuclei (Ramanna, 1974). In the colchicine-treated, diploid *A. cristatum*, secondary, supernumerary cleavage occurs following metaphase II (Tai, 1970).

In the present study, the relationship between nuclear division and cytokinesis, and the significance of supernumerary cytoplasmic cleavage in chromosome elimination will be evaluated.

MATERIALS AND METHODS

Fairway crested wheatgrass, and economically important plant, has been identified as *Agropyron cristatum* (L.) Gaertn. It is characterized as its low, leafy habit and broad spikes. Seeds of diploid ($2n=14$) *A. cristatum* were treated with 0.1% colchicine, and the progeny produced were numbered CB-9-85. The origin source of seeds was from Utah State University. This plant was found to have multipolar cell division. Seeds harvested from CB-9-85 were grown in the open field nurseries in summer. Some of the seeds produced vigorous plants; some, weak plants; and some failed to germinate. These plants are assumed to be the result of outcrossing. In the field, the developmental stages of the spikes are asynchronous and the stage of spike development often influence the success of pollination. The best material (spike) for cytology some from the middle of the flowering period. Florets from early or late blooming plant were often nonfertile. Young spikes were collected around 6 a.m. in

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June and fixed immediately in Newcomer's solution (6 isopropyl alcohol: 3 propionic acid: 1 petroleum ether: 1 acetone: 1 dioxane). Anther smears were used for the study of meiosis in pollen mother cell. Dissected out three anthers from spikelet under a dissecting scope and placed in center of a chemically clean slide and put a small drop of Belling's aceto-carmine on the top of the anthers. Squasned the anthers with a glass rod until the pollen mothers had been forced out. Removed debris and added cover glass and observed under low power. Then heated gently over an alcohol lamp, and pressed down the cover glass with thumb. Well prepared slides were examined under high power and sealed with paraffin. Pictures were taken by Zeiss universal microscope.

RESULTS

At late telophase I and telophase II, the pollen mother cell began its cytoplasmic cleavage. Cytoplasmic cleavage could proceed symmetrically around the entire cell, but also often asymmetrically (Figs. 1 and 2). After first meiotic division, two daughter cells were formed. Occassionally the cells were separated by a thin cytoplasmic connection (Fig. 1C). However, cytoplasmic cleavage might precede nuclear formation. As seen in Fig. 3, the process occurred in three-serial cell whose chromosomes remained separate. The two smaller cell contained 14 chromosomes each, and the larger one contained 30 chromosomes. It also showed that meiocytes with precocious division in meiosis I could advance to meiosis II stages, and secondary cytoplasmic cleavage could begin before the completion of first cytoplasmic cleavage. Furrowing process in this asynchronized cell involved twisting as well as contriction (a shearing force) instead of a progressive deepening as previously described (Esau, 1965).

Irregular cytoplasmic cleavage often occurred with multipolar cell division, lagging chromosomes, chromosome bridges and precocious division (Table 1). Chromosome bridges and lagging chromosomes were observed both in late anaphase I and II (Fig. 4). As shown in Fig. 4F, cytoplasmic cleavage occurred in telophase I that a chromosome bridge persisted in spite of the apparent termination of cytokinesis.

Occasionally daughter cells might have more than one nuclei in each cell. Extreme case came from 10 nuclei within a cell with 1 nucleus and 9 micronuclei. After meiosis I, supernumerary cytoplasmic cleavage happened and it might or might not follow nuclear division (Fig. 2). Evidence is that the number of cell in dyad meiocyte was not correlated to the

Table. 1. Chromosome behavior at anaphase I in F_1 progeny of CB-9-85

	Normal (%)	MCD* (%)	Fragements (%)	Laggards (%)	Bridges (%)	Precocious division (%)	Total
Plant number							
A-1-9	7(25.0)	18(64.3)	0	3(10.7)	0	0	28
A-1-10	15(26.3)	37(63.9)	1(1.8)	1(1.8)	3 (5.2)	0	57
A-1-27	4(12.1)	16(48.5)	0	8(24.2)	3 (9.1)	2(6.1)	33
A-1-31	12(24.5)	22(45.9)	3(6.1)	8(16.3)	3**(6.1)	1(2.1)	49
Total (mean, %)	38(22.7)	93(55.7)	4(2.4)	20(12.0)	9 (5.4)	3(1.8)	167

* Multipolar cell division.

** Chromosome bridge and fragement present in the same cell.

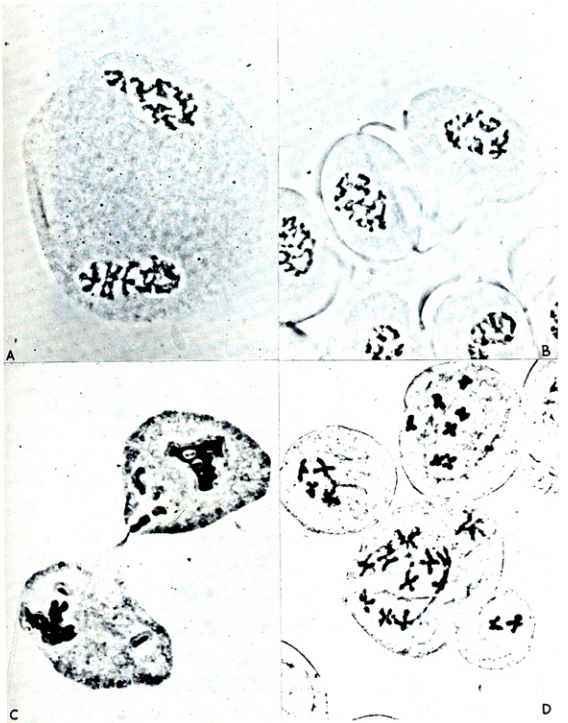

Fig. 1. Cytoplasmic cleavage after first meiotic division.
A-C. Symmetrical division (750×).
D. Asymmetrical division (750×).

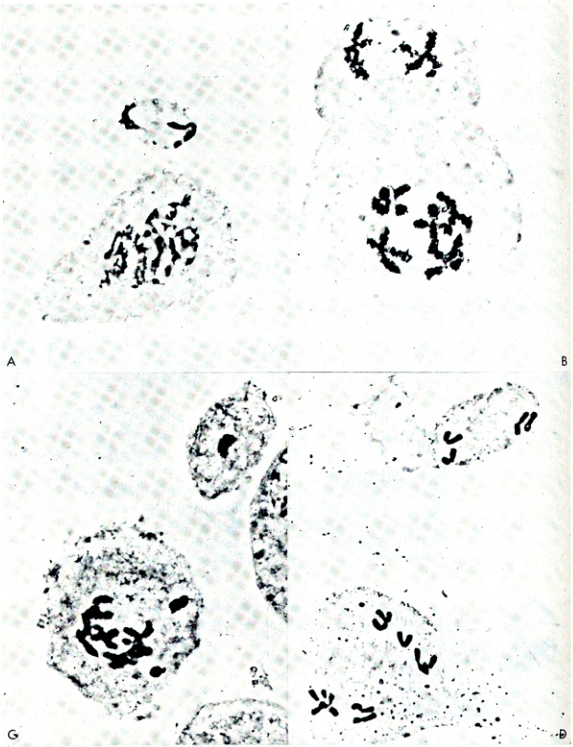

Fig. 2. Cells, originated from supernumerary cleavage of meiotic cell after metaphase II, contain different chromosomes.
A-B. Without chromatic separation (1200×).
C. Single chromosome (1600×).
D. 5-5 and 2-2 chromomatid separation at anaphase II (780×).

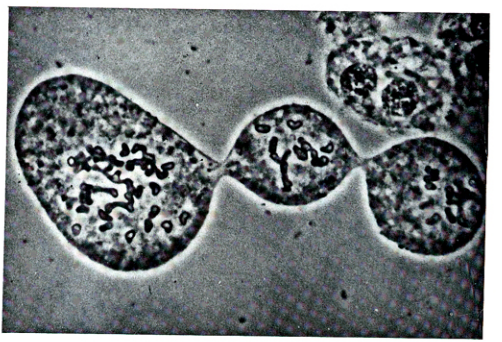

Fig. 3. Furrowing process in asynchronized cells (640×).

Table 2. The number of nuclei and the number of cells at dyad stage in the progeny of CB-9-85

		Number of cells produced				
		1	2	3	4	5
Number of nuclei produced	2(838)	—	838	—	—	—
	3(445)	—	437	8	—	—
	4(135)	—	126	7	2	—
	5(10)	—	8	1	1	—
	6(17)	—	13	4	—	—
	7(0)	—	—	—	—	—
	8(1)	—	1	—	—	—
	9(0)	—	—	—	—	—
	10(1)	—	1	—	—	—
Total	1447	0	1424	20	3	0

number of nuclei (Table 2). In most case where the number of cells produced was smaller than the number of nuclei produced. In spite of the number of chromosomes in each cells originated from same meiocyte could proceed secondary meiotic division and formed a quartet. A quartet contained four cells in tetrahedral configuration. But sometimes more than four cells in a quartet were often observed (Fig. 5 and Table 3).

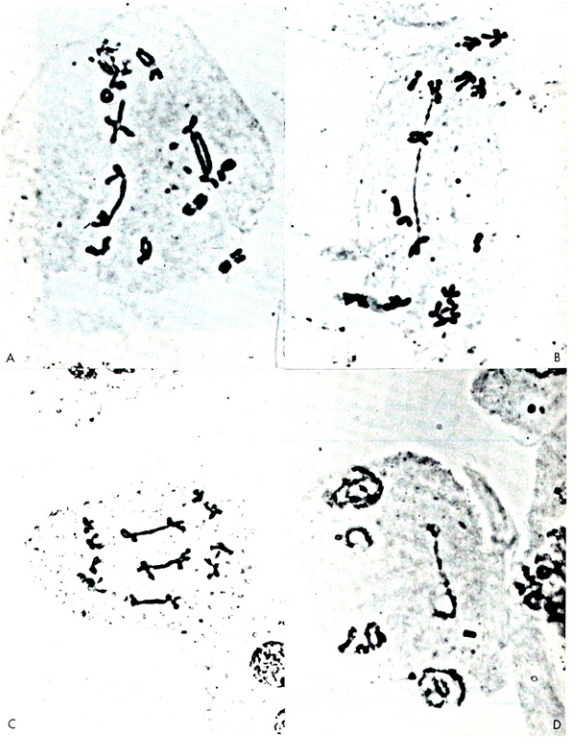

Fig. 4. Chromosome bridges, chromosome fragments and lagging chromosomes occurring at different stage of meiosis.
A-C. Chromosome bridges at anaphase I (725×).
D-F. Chromosome bridges at two-celled stage (950×).
G-H. Chromosome bridges at anaphase II (950×).

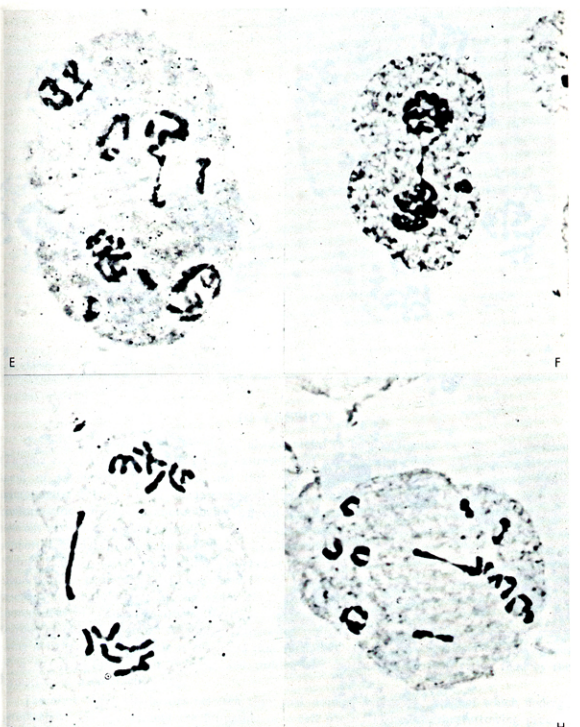

Fig. 5. Supernumerary microcells and micronuclei occurring in quartet (900×).

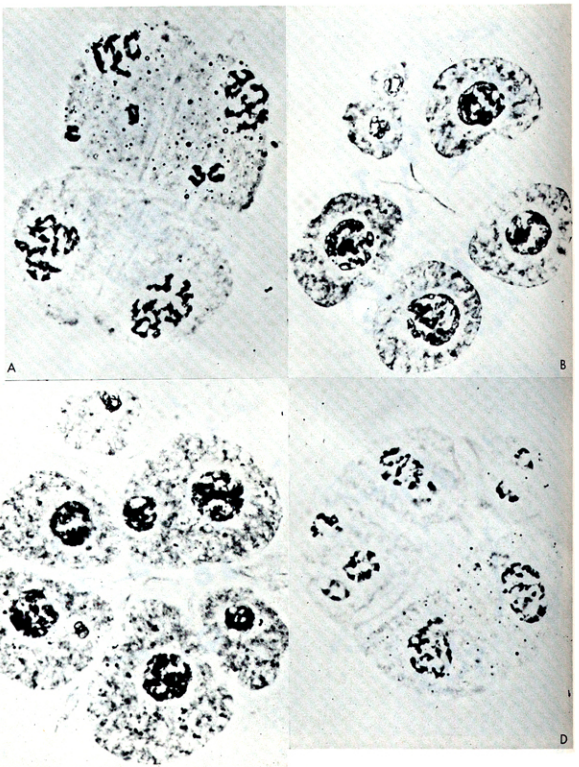
A
B
C
D

Table 3. The number of nuclei and the number of cells at quartet stage in the progeny of CB-9-85

		Number of cells produced						
		4	5	6	7	8	9	10
Number of nuclei produced	4(929)	929	—	—	—	—	—	—
	5(529)	452	77	—	—	—	—	—
	6(448)	400	26	12	—	—	—	—
	7(174)	135	39	—	—	—	—	—
	8(161)	151	8	1	1	—	—	—
	9(60)	57	3	—	—	—	—	—
	10(39)	34	5	—	—	—	—	—
	11(10)	6	4	—	—	—	—	—
	12(11)	9	1	1	—	—	—	—
	13(7)	7	—	—	—	—	—	—
	14(4)	3	—	1	—	—	—	—
	15(1)	1	—	—	—	—	—	—
	16(0)	—	—	—	—	—	—	—
	17(1)	1	—	—	—	—	—	—
	18(1)	—	—	1	—	—	—	—
Total	2378	2198	163	16	1	0	0	0

DISCUSSION

Cytoplasmic cleavage in animal cell always seems to be coordinated by the microtubules (Tamma *et al*, 1969). Margulis (1973) indicated that microtubules clearly underlie the development of asymmetric cell shapes, and that slow morphogenetic movement, which involves microtubular polymerization, tends to be colchicine sensitive. Moreover, that study stated that conformation changes in microtubular protein may account for the chemosensitivity of the nervous system of higher animals. The concept of microtubules as cellular skeletons has been supported by data from plant materials. In *Chlamydomonas* (Johnson and Porter, 1968) and in *Nassula* (Tucker, 1971), cytoplasmic cleavage is associated with a contractile ring of microfilaments formed perpendicular to the plane of the furrow. After telophase the microtubules are oriented in approimately in the same plane and within the ring of microfilaments. In zoosporogenesis of *Thraustochytrium* (Kazama, 1975), subplasmalemma microtubules crossed the furrow, at oblique angles. A similar furrowing process was observed in the present study, using a light microscope. It is suggested that furrowing cleaves the cytoplasm by the means of a shearing force, both twisting and pulling the daughter cells apart, rather than by the formation of a contractile ring.

There are many similarities between plant and animal meiocytes, both of which undergo cytoplasmic cleavage without the formation of a cell plate. Therefore, the furrowing process, as seen in microsporogenesis, is viewed as intermediate between the conventional types of cytokinesis cited to separate plants and animals. Perhaps furrowing, represents an efficient mechanism conserved through the otherwise divergent evolution of the two kingdoms.

After studying wall pattern in microsporogenesis of plants, Heslop-Harrison (1971) schemed

that the characteristic pathways from the initially pollen mother cell to quartet are principally related to the cleavage planes of the two meiotic division, and indicated that the furrowing process occurred in monocotyledon and cell-plate formation in dicotyledons. However, as early as 1916, Farr noted that presence of the furrowing process in numerous dicot genera including *Nicotiana*, *Primula*, *Tropelum*, *Ambrosia*, *Chrysanthenum*, *Helianthus* and others. From this study, it shows that both furrowing process and cell plate formation may occur in microsporogenesis of *Agropyron cristatum*. Further study on ultrastructure of meiocyte development is required.

Supernumerary cytoplasmic cleavage often occurs in cells with multipolar cell division (Tai, 1970). It can happen after first meiotic division or second meiotic division (Chen, 1975). First supernumerary cytoplasmic cleavage may or may be not related to the numbers of chromosome grouping at metaphase I and give rise to microcells with one to several dyad chromosomes. Sister microcells like normal meiocytes can advance to anaphase II but more or less asynchronized. This may indicate that the disjunction of chromosome always related to the presence of chromosome, even only one. Kinetochore plays an important role in this phenomenon (DuPraw, 1970). Secondary supernumerary cleavage occurs after anaphase II and produce a quartet containing more than four cells. The number of cells per quartet is apparently depended upon the frequency of supernumerary cytokinesis after meiosis II. Pollen grains developed from microcell in quartet contain regular and irregular number of chromosomes. Irregular chromosome number pollen may be viable and functional for pollination and fertilization, and it will result in the change of chromosome number in its progeny.

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