

Cytomixis in *Agropyron cristatum*

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An *Agropyron cristatum* plant (CB-9-41), crested wheat grass, and its vegetative clones have been identified that contain pollen mother cells that have a gain or a loss in chromatin (DNA). CB-9-41 was identified during the course of an experiment to determine the effectiveness of colchicine on the doubling of the chromosome complement. The seeds that produced this plant were presoaked and then treated with a 0.1% aqueous solution of colchicine for 12 h. All stages of meiosis were studied in the original colchicine-treated plant and three vegetative clones that were obtained 17 years later. Approximately 40% of the pollen mother cells had meiotic irregularities. These irregularities were caused by multipolar meiosis (23%), precocious separation of bivalents at metaphase (8%), inversions (6%), and cytomixis (11%). The gain or loss of chromatin occurred as a result of cytomixis.

Key words: crested wheat grass, extragenomic chromatin, multipolar meiosis, colchicine.

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Une plante d'agropyre à crête, *Agropyrum cristatum* (CB-9-41), et ses clones ont été identifiés et se sont avérés posséder des cellules-mères de pollen avec gains ou pertes de chromatine (ADN). Cette lignée CB-9-41 a été identifiée au cours d'une expérience visant à déterminer l'efficacité de la colchicine sur le doublement des garnitures chromosomiques. Les graines à l'origine de cette lignée furent soumises à trempage à l'eau, puis traitées à la colchicine en solution aqueuse à 1% durant 12 h. Les divers stades de la méiose ont été étudiés chez la plante-mère traitée à la colchicine, ainsi que chez trois de ses clones végétatifs obtenus 17 années plus tard. Environ 40% des cellules-mères de pollen ont présenté des irrégularités méiotiques. Ces irrégularités comportaient une méiose multipolaire (23%), une séparation hâtive des bivalents à la métaphase (8%), des inversions (6%) et de la cytomixie (11%). Les pertes ou les gains en chromatine étaient liés à la cytomixie.

Mots clés: agropyre à crête, chromatine extragenomique, méiose multipolaire, colchicine.

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Introduction

Tai and Dewey (1966) treated 5000 diploid *Agropyron cristatum* L. Gaertn., crested wheat grass, seeds with colchicine to double the chromosome complement. The treatment produced some tetraploid sectors as shown by morphological and cytological data (Tai and Dewey 1966). Meiotic studies of the treated but undoubled plants revealed that the colchicine may have had additional effects, such as multipolar meiosis in some of the treated diploid plants (Tai and Dewey 1966). This study describes another plant (CB-9-41) and its vegetative clones in which cytomixis has occurred.

Cytomixis is the phenomenon of extrusion of chromatin from one cell into the cytoplasm of an adjoining cell (Gates 1911). Cytomixis was first observed by Kornicke (1901) in *Crocus* and then by Gates (1911) who studied the pollen mother cells (PMCs) of *Oenothera gigas* and coined the term cytomixis. Since that time, many more plants have been identified that have undergone cytomixis: *Andropogon*, *Digitaria*, *Echinochloa*, *Miscanthus*, *Panicum*, *Paspalum*, and *Sorghastrum* (Church 1929); *Thespesia* (Youngman 1931); *Oryza sativa* (Nandi 1937); *Secale cereale* (Muntzing and Prakken 1942); *Aloe*

(Mendes and Rijo 1951); *Lilium* (Cooper 1952; Noda 1971); *Gossypium* (Sarvella 1958); *Tauschia nudicaulis* (Bell 1964); *Lotus* (deNettancourt and Grant 1964); *Triticum* (Rana 1965); *Curcubita* and *Lycopersicum* (Welling 1965); *Aegilops* × *Triticum* hybrids (Shkutina and Kozlovshaya 1974); *Hemerocallis* (Narain 1979); *Gloriosa* (Narain 1980); and *Zea mays* (Peeters et al. 1985).

This study was undertaken to determine the frequency and characteristics of the PMCs that resulted from cytomixis in CB-9-41 and the possible mechanism of its origin.

Materials and methods

Open-pollinated seed was used from a breeding nursery at Utah State University, Logan, Utah, of diploid *A. cristatum* cv. Fairway. A total of 5000 seeds were treated. Approximately 2500 seeds were soaked in distilled water for 24 h and the remaining half were not presoaked. Seeds were placed in petri dishes (100/dish) on germination blotters saturated with a 0.0, 0.1, 0.2, 0.4, or 0.8% (w/v) aqueous solution of colchicine. The duration of treatments was 12, 24, 48, 72, or 96 h. The seeds were then removed and germinated on blotters saturated with tap water. Seedlings were reared in the greenhouse and then transplanted to the field; data were taken after 1 year. Spikes from each plant were fixed in Carnoy's fluid for at least 24 h. Observations were made on all stages of meiosis by using standard acetocarmine squash techniques.

Routine cytogenetic examination of the plants revealed one plant (CB-9-41) from the 12-h 1% solution treatment, which contained

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PMCs with a gain or a loss in chromatin. Feulgen staining verified the extra chromatin as DNA. The original observation was made in 1963 and only 80 PMCs were counted at that time. Each year since that time this plant has been propagated vegetatively. In this study, meiosis was observed in the PMCs from spikes from the original plant and spikes from three vegetative clones obtained from Dr. Douglas R. Dewey (Utah State University, Logan, Utah) in 1980. Approximately 3000 PMCs were observed for each vegetative clone.

Male fertility was estimated from the percentage of pollen that stained black in an aqueous solution of I_2 -KI (Hauser and Morrison 1964). Female fertility was measured by the percentage of seed set of five spikes from five different plants which were open pollinated.

Results

Meiosis proceeded normally in the control plants as well as in most of the PMCs of CB-9-41. The 14 chromosomes typically form seven bivalents that align themselves on a single equatorial plate followed by a seven-seven bipolar anaphase I disjunction and a cytoplasmic cleavage that gives rise to two daughter cells each with seven chromosomes. Meiosis is synchronized so that sister cells proceed simultaneously through metaphase II, anaphase II, telophase II; typically, a four-celled sporad is formed.

In 11% of the early prophase cells, a small amount of Feulgen positive material (DNA) was observed toward the periphery of the cell in addition to a normal nucleus. Usually this extra chromatin was less condensed than the normal nucleus. In some cases more than one nucleus could be found. At diakinesis, 22.1% of the cells contained seven normal bivalents with late condensing chromatin (Table 1). Also during diakinesis, 324 of the 1751 cells counted showed a tendency for the chromosomes to form two, three, or four groups, indicating that a multipolar division might ensue (Table 1).

At metaphase I, 6.6% of the cells contained extra chromatin. In most of the cells it appeared as early prophase chromatin (Fig. 1), but occasionally the extra material appeared the same as normal bivalents (Fig. 2). Extra bivalents (8, 9, or 10) were observed in 12 of the cells that contained extra chromatin. However, as few as five or six bivalents were observed in 28 of the 2370 PMCs observed (Table 1). In 21% of the cells observed at metaphase I, multipolar divisions occurred with the chromosomes forming two, three, or four groups, i.e., a group of four chromosomes plus a group of three chromosomes or a group of three chromosomes plus another group of three chromosomes plus one chromosome, etc. The observation of aligned univalents indicated the early separation of bivalents in 8.5% of the cells (Table 1).

The irregularities that were found in anaphase I were a reflection of what was observed at earlier stages of meiosis. Extra chromatin (4.3%) (Fig. 3) and multipolar divisions (27.8%) were seen as well as the formation of chromatin bridges and acentric fragments in 90 of the 1307 cells (Table 1).

Cytokinesis occurred at the end of the first meiotic division. Extra chromatin was observed throughout the second division of meiosis. Probably because of multipolar meiosis, the two sister cells proceeded through the second meiotic divisions nonsynchronously (Table 1).

The same types of irregularities that occurred during the first meiotic division were observed during metaphase II and anaphase II. Extra chromatin was found in 12.3% of the cells at metaphase II (Fig. 4). Sixteen sporads were found at metaphase II, which contained six chromosomes in one cell and seven in the sister cell (Table 1). Evidence of cytotoxicity was found in 5.4% of the cells at anaphase II (Table 1). Chromatin bridges

TABLE 1. Meiotic chromosome behavior of a colchicine-treated *Agropyron cristatum* and three vegetative clones obtained 17 years later

Stage	Observations	No. of cells	%
Diakinesis	Normal	1001	57.2
	MPD ^a	324	18.5
	Extra	387	22.1
	Loss	39	2.2
	Total	1751	
Metaphase I	Normal	1487	62.7
	MPD	496	21.0
	Extra	157	6.6
	Loss	28	1.2
	Early separation ^b	202	8.5
	Total	2370	
Anaphase I	Normal	791	60.7
	Bridge	90	6.9
	MPD	363	27.8
	Extra	57	4.3
	Loss	6	0.5
	Total	1307	
Metaphase II	Normal	792	58.2
	Nonsynchronous ^c	98	7.2
	Extra	167	12.3
	Loss	16	1.2
	MPD	288	21.1
	Total	1361	
Anaphase II	Normal	825	59.3
	Bridge	81	5.8
	MPD	411	29.5
	Extra	50	3.6
	Loss	25	1.8
	Total	1392	
Sporad	Normal	1028	47.0
	Micronucleated ^d	296	13.5
	Supernumerary ^e	642	29.3
	Different sizes ^f	223	10.3
	Total	2189	

^aMultipolar cell division.

^bEarly separation of bivalents at metaphase I.

^cBoth cells not at the same stage, i.e., metaphase II and anaphase II

^dOne to six micronuclei per sporad

^eFive to eight cells per sporad.

^fDifferent-sized cells composing the sporad, i.e., five large cells plus three smaller cells or one large cell plus five smaller cells.

with acentric fragments were detected in 81 of the 1393 (5.8%) anaphase II cells (Table 1). Multipolar divisions were more evident (29.5%) at anaphase II than at any other stage of meiosis. A supernumerary cytoplasmic division occurred in many of the abnormal cells, which gave rise to microcells containing one to six chromosomes (Fig. 5).

The sporad stage reflected the abnormal behavior seen earlier. One to six micronuclei were seen in 13.5% of the sporads with each individual cell containing one to three micronuclei (Fig. 6). The number of cells per sporad was four to six depending on the frequency of supernumerary cytokinesis. Sporads containing more than four cells were found in 642 of the 2189 cells observed. Different-sized cells were observed in 10.2% of the sporads. These sporads ranged from five large plus one small cell to five small cells plus one large one (Table 1). The cytological behavior of the original plant and the three

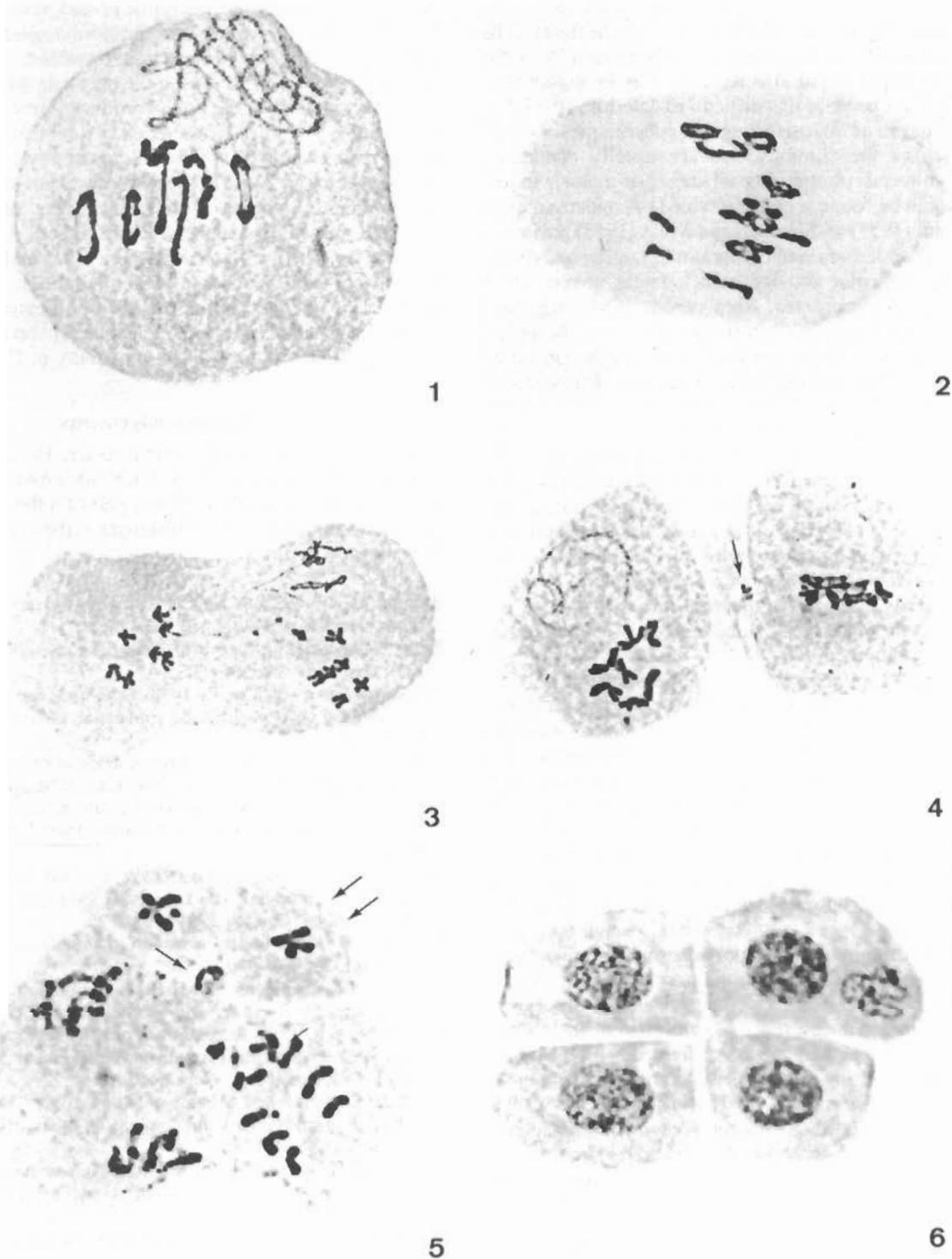


FIG. 1. Metaphase I with seven bivalents and strands of late condensing chromatin. $\times 850$. FIG. 2. Metaphase I with 10 bivalents. $\times 850$. FIG. 3. Anaphase I with a seven-seven segregation plus extra chromatin. $\times 800$. FIG. 4. Metaphase II with one cell containing extra chromatin plus a chromosome trapped in between the two cells (arrow). $\times 775$. FIG. 5. A PMC showing the results of supernumerary cleavage (micronuclei shown with a single arrow and microcell shown with double arrows). $\times 825$. FIG. 6. Quartet stage showing a normal quartet with an extra strand of chromatin. $\times 825$.

vegetatively propagated clones was essentially the same and thus data were combined (Table 1).

Seventy percent of the pollen stained black in I_2 -KI and thus was presumed to be viable. Mature plants were fertile with an average of 205 spikelets per five heads, 221 seeds per five heads, and 1.1 seeds per spikelet.

Discussion

Sixty percent of the cells in CB-9-41 behaved normally; the remainder exhibited various amounts of chromatin, multipolar meiosis, early separation of bivalents at metaphase I, chromatin bridges with acentric fragments, nonsynchronous divisions at anaphase II, micronuclei, supernumerary sporads, and variation

in the size of the cells at the sporad stage. Extra chromatin appeared at all stages of meiosis in 9% of the total cells observed. The loss of chromatin was only observed in 2% of the cells. The true proportion of affected cells may be higher than recorded in Table 1 because it is difficult to determine the DNA content at all stages of meiosis using these techniques.

During meiosis the chromosomes are usually condensed equally and uniformly, but during all stages of meiosis in this study cells could be found which contained uncondensed extra chromatin. Jain (1957) and Holden and Mota (1958) attributed the differential condensation of chromatin in *Lolium* and *Avena* to the lack of nucleolar activity, which might govern DNA synthesis of the chromosomes. Rees (1958, 1961) suggested that it may be under genetic control. In some cases, however, the extra chromatin material appeared as normally condensed chromosomes for that particular stage of meiosis. The source of the extra chromatin is possibly from surrounding cells that were shown to have lost some chromatin (Table 1).

Some cytologists consider cytomixis an extremely abnormal phenomenon. Gates and Rees (1921), Woodworth (1931), Sax (1937), Jacob (1941), Sarvella (1958), Welling (1965), Bobak and Herich (1978), and Narain (1980) suggest that cytomixis is due to faulty techniques and environmental stresses while handling the living material such as temperature changes, fixation, chemicals, mechanical pressure, disease, insects, and physiological abnormalities. All of these stress factors could have induced cytomixis in CB-9-41.

In this study, cytomixis may have been induced by colchicine. Gates and Rees (1921), Beadle (1932), and Kamra (1960) have suggested that cytomixis occurs because the cell wall fails to form at premeiotic mitosis. Abnormal cell wall formation has been observed when colchicine has been applied. Colchicine is known to affect the microtubules, which are needed for cell wall formation (Hardham 1982). Narain (1980) suggested that the amount of material extruded from one cell into another, i.e., either a short strand of chromatin or whole chromosomes, could depend on the nature and number of protoplasmic connections of the affected cell. Once cytomixis occurs, it can be perpetuated indefinitely through asexual propagation, as shown by the stability of this plant material over the past 17 years.

The effect cytomixis has on the process of meiosis is compounded owing to the presence of multipolar meiosis where multipolar meiosis is a spontaneous or induced spindle apparatus abnormality resulting in genome separation during meiosis (Li and Tu 1947; Thompson 1962; Tai 1970). Tai (1970) proposed that multipolar meiosis occurs via a genome-specific microtubule-organizing center (cell organelles that govern chromosome migration and cytokinesis (Pickett-Heaps 1969)) that possibly provides an evolutionary mechanism for haploidization in higher plant polyploids.

Multipolar meiosis could be observed during all stages of meiosis, and as meiosis proceeded, the number of affected cells seemed to accumulate (Table 1). At diakinesis and metaphase I, the bivalents appeared to form two or more groups, and these groups with their associated spindle acted independently of each other. The early separation of bivalents at metaphase I may be attributed to this independence. It was during the second division that the separate action of the spindles was manifested, i.e., one cell often remained at metaphase II while its sister cell proceeded towards telophase II. The occurrence of chromatin bridges and acentric fragments during both anaphase I and II is evidently the result of an inversion (Tai and Dewey 1966).

The sporad stage was the most difficult to analyze. Many of the sporads seemed abnormal, therefore making it difficult to

distinguish whether the micronuclei and supernumerary cells were the results of cytomixis or multipolar meiosis. We assume that the gain and loss of chromatin remained at approximately the same level as it had during the previous stages.

Seventy percent of the pollen produced may be functional as indicated by staining. However, it is doubtful that the smaller pollen grains would be functional gametophytes. The reduction in pollen viability is due to the cytological abnormalities seen in these plants. Stebbins (1958), however, pointed out that although meiotic irregularities exist, genetic factors may also account for sterility. Even with these abnormalities the mature plants were fertile as shown in the production of 1.1 seeds per spikelet. In untreated checks of open-pollinated diploid *Agropyron cristatum*, 2.55 seeds per spikelet were observed in a breeding nursery at Utah State University in 1964.

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