A Polyhaploid Clone of *Bromus inermis* Leyss with Chromosome Fragments

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An octoploid chromosome number of 56 has commonly been reported in *Bromus inermis* Leyss although different chromosome numbers constituting a ploidy series of 28, 42, 56 and 70 have been reported (Myers 1947). At the octoploid level, chromosome anomalies such as aneuploidy (Sigurbjornsson *et al.* 1958) and accessory fragments (Hill and Myers 1948, Nielsen 1955) in addition to the regular complement of 56 chromosomes have been reported.

The fertile polyhaploid plant found by Elliott and Wilsie (1948) was confirmed as a tetraploid with a somatic complement of 28 and a meiotic set of 14 bivalents by Knobloch (1953). The chromosomes were reported to pair regularly at meiosis. The possible origin of the tetraploid could have been either by doubling of the chromosomes in some ancestral diploid, or through somatic reduction of an octoploid (Knobloch 1953). Chromosomal fragments have never been reported for the tetraploid. However, during the course of an investigation of different ploidies in the species, additional fragments to the normal chromosome complement were frequently found in the root tip cells of one of the polyhaploid ramets. The primary objective of the present report was to describe the irregular behaviour of the chromosomes at mitotic division of the root tip cell from this particular ramet, and to detect the possible origin of the fragments.

Materials and methods

The polyhaploid plant used in the present study was originally obtained from the Iowa Agricultural Experiment Station and was an asexual derivative from the plant designated 554-39 which was previously reported by Elliott and Wilsie (1948). The ramets of the polyhaploid plant were space-planted at the Kingman Farm, University of New Hampshire, for a number of years and were well established.

Young root tips were collected from potted ramets in the greenhouse. The root tips were pretreated in 0.002 M 8-hydroxy-quinoline for 3 hours and then fixed in Carnoy's solution overnight. Staining of chromosomes was done according to the Feulgen method as described by Darlington and LaCour (1962). Un-pretreated materials were also included for observations at mitotic anaphase.

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Results and discussion

A fragment was frequently observed in root tip cells of a particular polyhaploid bromegrass ramet under study (Fig. 1). In some instances, the fragment number varied from two to three per cell at metaphase (Figs. 2 and 3). Occasionally, minute fragments were also observed (Fig. 4).

In an attempt to detect the possible origin of the fragment, the karyotypes of the ramet with fragment and a normal polyhaploid ramet grown adjacently were compared, using the same cytological technique. A somatic chromosome number of 28 was found for the normal (Fig. 5A) and $28+(1)$ for the ramet with fragment (Fig. 5B) in the majority of cells observed. Apart from the detachment of the satellite from one of the SAT-chromosomes in Fig. 5B, the two ramets had essentially similar karyotypes: one pair of satellite chromosomes, five pairs of metacentric, five pairs of submetacentric and three pairs of subtelocentric chromosomes. Two large satellites attached to the distal end of the pair of subtelocentric chromosomes were clearly observed (Fig. 5A), while the two minute satellites reported by Wilton

Figs. 1-4. 1, root tip mitotic complement of $4x$ *Bromus inermis* with 28 chromosomes and one fragment (indicated by arrow). 2, somatic metaphase showing two fragments. 3, early metaphase showing three fragments. 4, metaphase with twenty-three chromosomes, one fragment and two minute fragments (indicated by arrows).

Fig. 5. A, karyotype from normal 4x clone. B, karyotype from 4x clone with one fragment.

(1965) were not visible in our preparations. The fragment shown in Fig. 5B was comparable in length (approximately $1\ \mu$) with the satellite of the SAT-chromosome shown in Fig. 5A. The satellite of one of the SAT-chromosome was apparently the source of origin for the fragment.

There are several possibilities for the detachment of the whole satellite from the parent chromosome. Mitotic disarticulation at the weak secondary constriction was reported to be a frequent case through chemical treatment and squashing in the genus *Bromus* (Markarian and Schulz-Schaeffer 1958). However, use of the same cytological technique with the two materials might exclude this possibility. Another possibility is that the satellite was actually connected to the short-arm by means of an attenuated slender thread though invisible in our preparations. However, the distance between the satellite and the parent chromosome in some cases decreased this possibility (Figs. 1–3).

Extensive observations made on the cytological behaviour of the SAT-chromosome during mitotic division of unpretreated root tips revealed that division of the SAT-chromosome was significantly delayed at anaphase in materials with fragments. Approximately fifty per cent of the cells at anaphase exhibited irregular chromosome separation. In some cases the lagging satellite divided eventually at late anaphase (Fig. 6). An attenuated thread connected to each pole was clearly observed. Fig. 7 shows the daughter halves of the satellite lying closely parallel, but they might be reversely oriented with respect to their homologous segments. Despite a delay, the satellites separated and were incorporated normally into the daughter nuclei at the poles (Fig. 8). If the separation of daughter satellites was significantly delayed, the poleward movement was so tensed that finally a break occurred (Fig. 9), and the daughter satellite was disconnected from the short arm of one of the SAT-chromosomes. Consequently, the daughter satellite was detached and remained in the cytoplasm as a fragment at later stages of the mitotic division (Fig. 10). Mitotic instabilities such as lagging chromosomes were also encountered in some cells (Fig. 11). This could reduce the somatic chromosome number to less than 28 as shown in Fig. 4.

During our observations at metaphase, there was no evidence for precocious divisions of the satellite and its consequent detachment from the SAT-chromosomes as described by Shirato in *Brodiaea uniflora* Engl (1966). As illustrated above, the fragment in the polyhaploid clone, in which one of its SAT-chromosomes underwent irregular division at mitotic anaphase, most probably originated from an anaphasic break at the secondary constriction of the SAT-chromosome due to the poleward movement. Marchant and Brighton described similar results in *Ranunculus ficaria* (1971). They claimed that the unusual and irregular behaviour of an excessively enlarged satellite region on one of the four SAT-chromosomes at anaphase

Figs. 6-11. 6, lagged daughter satellites connected to each pole by attenuated slender threads. 7, lagged daughter satellites lying closely parallel showing their reversed orientation. 8, late telophase showing the daughter satellites incorporated into the daughter nuclei. 9, the daughter satellites remain attached, showing breakage at one end while the other daughter satellite still being connected to the pole by a slender thread. 10, a lagged fragment and the other daughter satellite being pulled close to one pole. 11, anaphase showing lagged chromosomes and three fragments.

had given rise to fragments of various sizes. Misdivision, or nondisjunction of daughter fragments at anaphase, could cause variation in number of fragments in subsequent generations of cell divisions.

The origin of the minute fragments remains obscure (Fig. 4). They might arise from anaphasic breakage at the secondary constriction of the SAT-chromosome with minute satellites which were not visible in our preparation. The polyhaploid ramet was maintained only by vegetative propagation, therefore the occurrence of fragments cannot be attributed to irregular meiosis through translocation, inversion or deletion.

No morphological differences were found between the polyhaploid ramets with

and without the fragment. Moreover, there were no detrimental effects on seed set on the ramet with the fragment, and selfed progenies were obtained easily.

Summary

An additional fragment frequently appeared in the root tip cells from a polyhaploid clone of smooth brome grass (*Bromus inermis* Leyss). Occasionally, two or three fragments could be observed.

Comparison of karyotypes between ramets with and without a fragment revealed that the fragment may have originated from the satellite which was detached from one of the SAT-chromosomes. The division of the SAT-chromosomes was considerably delayed at anaphase in the unpretreated root tip from the ramet with the fragment. In most cases the lagged satellite eventually divided and incorporated into the daughter nuclei. However, in many divisions the separation of the daughter satellites was so much delayed that, as a result of the poleward movement, a break occurred between the daughter satellite and one of the parent chromosomes. The detached daughter satellite later became a fragment. Misdivision, lagging or non-disjunction of the daughter fragment could explain the different number of fragments in subsequent generations of cell divisions.

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