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# Fate of multicentric and ring chromosomes induced by a new gametocidal factor located on chromosome 4M$^g$ of *Aegilops geniculata*

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**Abstract**

A new gametocidal (Gc) factor was identified on chromosome 4M$^g$ of *Aegilops geniculata* Roth. When transferred to Chinese Spring wheat, monosomic and disomic *Triticum aestivum–Ae. geniculata* chromosome 4M$^g$ addition plants undergo regular first and second meiotic divisions. Male gametogenesis in disomic 4M$^g$ addition plants also is normal. However, chromosome breakage and anaphase bridges were observed at ana/telophase of the first (29%) and second (11%) pollen mitosis in monosomic 4M$^g$ addition plants. Gc-induced multicentric and ring chromosomes can be transmitted to the offspring and initiate breakage fusion bridge (BFB) cycles in dividing root tip meristem cells of the derived sporophytes. The fate of multicentric and ring chromosomes was analyzed in root meristems at different time intervals after seed germination. The majority of the BFB cycles ceased about 32 days after germination. Broken chromosome ends were healed either by the fusion of a centric and an acentric fragment forming terminal translocation chromosomes or as deficiencies or telocentric chromosomes. Lack of cytologically detectable telomeric repeats at the stabilized newly broken termini suggests that chromosome healing by addition of telomeric repeats may be a gradual process.

## Introduction

Gametocidal (Gc) factors have been identified in several diploid and polyploid species of the genus *Aegilops* (for review, see Endo 1990). When transferred to wheat, *Triticum aestivum* L. (2n = 6x = 42, AABBDD) or *T. turgidum* ssp. *durum* Desf. (2n = 4x = 28, AABB), disomic Gc chromosome addition plants have regular meiotic divisions and gametogenesis. Meiosis also is normal in plants monosomic for a Gc chromosome. However, chromosome breakage occurs at the G1 stage of the first postmeiotic interphase mainly in gametophytes lacking the Gc chromosome (Finch *et al*. 1984, King & Laurie 1993, Nasuda *et al.* 1998).

Several Gc systems have been described (Endo 1982, Finch *et al.* 1984, Tsujimoto & Tsunewaki 1984, 1985, Endo 1985a, 1985b, 1988, Tsujimoto & Tsunewaki 1988, Tsujimoto 1994, 1995). Strong Gc factors induce extensive chromosome breakage which results in non-functional gametes and, thus, in the exclusive transmission of the Gc chromosome to the offspring (Nasuda *et al*. 1998). A weak Gc factor induces lower levels of chromosome breakage that

can be tolerated because of the polyploid nature of bread wheat, and results in the formation of functional gametes (Nasuda *et al.* 1998). If chromosome breakage is followed by the addition of telomeric repeats, cytologically stable deficiencies are produced and can be recovered in the offspring of those plants (Werner *et al.* 1992, Tsujimoto 1993). This system was used to produce a series of deficiencies in common wheat (Endo & Gill 1996), rye (Endo *et al.* 1994, Kynast *et al.* 1998), and barley (Endo *et al.* 1998).

Here we report on the identification of a new Gc factor located on chromosome $4M^g$ of *Ae. geniculata* Roth ($2n = 4x = 28$, $U^gU^gM^gM^g$) which, when transferred to common wheat, causes moderate chromosome breakage mainly in gametophytes lacking the Gc factor. As a result, multicentric and ring chromosomes are produced that initiate breakage fusion bridge cycles (BFB), which can persist in the derived sporophytes. The fate of multicentric and ring chromosomes and the process of chromosome healing was analyzed in root tip meristems at different time intervals beginning at germination.

## Materials and methods

### *Plant material*

During the development of a set of *T. aestivum* cv. 'Chinese Spring'–*Ae. geniculata* (accession TA2899) chromosome addition lines, progenies from plants monosomic for chromosome $4M^g$ showed a high frequency of chromosomal instability including multicentric, ring, acrocentric, and telocentric chromosomes (Friebe *et al.* 1999). To further analyze this phenomenon, meiotic chromosome behavior and male gametogenesis was analyzed in plants monosomic and disomic for chromosome $4M^g$. Furthermore, chromosomal constitutions of 154 plants derived from selfing a monosomic $4M^g$ plant were determined and plants with multicentric and ring chromosomes were identified. The fate of the induced multicentric and ring chromosomes was analyzed in 15 plants at different time intervals after germination. Seedlings were transplanted into a mixture of equal volumes of soil and vermiculite 6 days after germination. Plantlets were grown at 12–14°C under short day (10-h day, 14-h night) conditions with high relative humidity and an ample supply of water and nutrients for good vegetative growth. Root tips were harvested 3, 10, 32, 58, 84, and 101 days after germination.

### *Chromosome analysis*

Root tips were pretreated in ice–water for 24 h and fixed in ethanol and glacial acetic acid (3 : 1), followed by Feulgen staining. Meiotic metaphase I pairing was analyzed in pollen mother cells (PMCs). First and second pollen mitoses were analyzed in plants monosomic and disomic for chromosome $4M^g$ as described by Nasuda *et al.* (1998). The centromeric DNA probe pRCS1 (Dong *et al*. 1998) and the telomeric DNA probe pAtT4 (Richards & Ausubel 1988) were digoxigenin-labeled and used to identify centromeres and telomeres in aberrant chromosomes by sequential fluorescence *in-situ* hybridization (FISH) to squash preparations of mitotic metaphase cells. The hybridization stringency was 63% for pRCS1 (37°C, 2 × SSC, 30% formamide) and 75% for pAtT4 (37°C, 2 × SSC, 50% formamide), respectively (Bolton & McCarthy 1962). Post-hybridization washes were at stringencies of 68% and 80% (42°C). Signals were detected sequentially using FITC-conjugated sheep–antidigoxigenin antibodies (Roche Molecular Biochemical, Indianapolis, Indiana, USA) according to the protocol of Leitch *et al.* (1991). Fluorescence signals were amplified using FITC-conjugated rabbit–antisheep antibodies (Vector Laboratories, Inc., Burlingame, California, USA) according to the manufacturer's recommendations. pAtT4 signals were further amplified using FITC-conjugated swine–antirabbit antibodies (Dako Corp., Carpinteria, California, USA) according to the manufacturer's recommendations. Signals were visualized using an Olympus BH-2 microscope equipped for phase contrast and epifluorescence. Images were captured with a SPOT CCD camera using the appropriate SPOT 2.1 software (Diagnostics Instruments, Inc., Sterling Heights, Michigan, USA) and processed with PhotoShop 4.0 software (Adobe Systems Inc., San Jose, California, USA). Images were printed on a Kodak ds 8650 PS Color Printer.

## Results

Meiosis was normal in plants disomic for chromosome $4M^g$ and 22 bivalents (22″) were observed at

metaphase I in all 75 PMCs analyzed. Monosomic 4M$^g$ addition plants also had no meiotic irregularities and chromosomes paired in the form of 21 bivalents plus one univalent ($21'' + 1'$, 72 PMCs) (Figure 1a) or as 20 bivalents plus three univalents ($20'' + 3'$, 3 PMCs) at meiotic metaphase I.

The first and second pollen mitosis in disomic chromosome 4M$^g$ addition plants also were normal (Table 1). However, in monosomic 4M$^g$ addition plants, chromosome breakage and anaphase bridges were observed at ana/telophase of the first pollen mitosis in 29% of the gametophytes (Table 1, Figure 1b). These plants had a lower frequency (11%) of aberrant ana/telophases in the second pollen mitosis (Table 1, Figure 1c).

Chromosomal constitutions were determined in root tip meristems of 154 plants derived from self-pollination of a monosomic 4M$^g$ addition plant (Table 2). Seventeen plants (11%) had 44 chromosomes, indicating the presence of two 4M$^g$ chromosomes.

*Table 1.* Percent aberrant ana/telophases with fragments and/or bridges at first and second pollen mitoses in disomic (DA) and monosomic (MA) chromosome 4M$^g$ addition plants.

| | Normal | Aberrant |
|---|---|---|
| **DA4M$^g$** | | |
| First pollen mitosis | 46 | 0 |
| Second pollen mitosis | 63 | 0 |
| **MA4M$^g$** | | |
| First pollen mitosis | 62 | 25 |
| Second pollen mitosis | 67 | 8 |

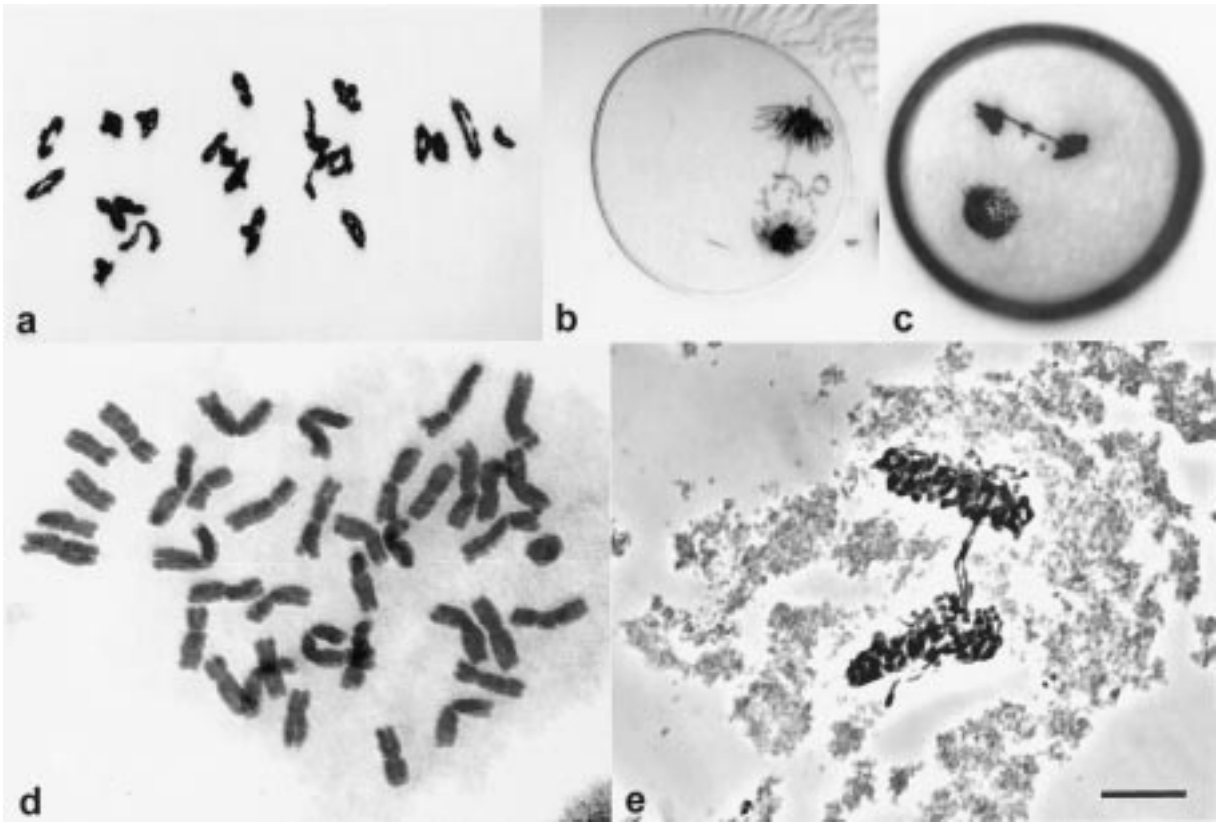


*Figure 1.* Meiotic metaphase I pairing and first and second pollen mitoses of a plant monosomic for chromosome 4M$^g$: (**a**) meiotic metaphase I showing regular bivalent pairing of wheat chromosomes and chromosome 4M$^g$ as a univalent; (**b**) ana/telophase of the first pollen mitosis with chromosome fragments and bridge formation; (**c**) ana/telophase of the second pollen mitosis with a chromosome fragment and bridge; (**d**) mitotic metaphase of a plant with one ring chromosome; (**e**) mitotic ana/telophase of the same plant shown in (**d**) with a double chromatid bridge indicating an active BFB cycle. Bar = 10 $\mu$m.

*Table 2.* Chromosome constitutions of 154 plants derived from self-pollination of a monosomic 4M$^g$ addition plant.

| Chromosome constitution | Number of plants | Percent of total |
|---|---|---|
| 41 | 3 | 1.9 |
| 42 | 37 | 24.0 |
| 43 | 25 | 16.2 |
| 44 | 13 | 8.4 |
| 36 + d + tr + r + t | 1 | 0.6 |
| 39 + d | 1 | 0.6 |
| 39 + d + d | 1 | 0.6 |
| 40 + d | 23 | 14.9 |
| 40 + d + r + a | 1 | 0.6 |
| 41 + d | 9 | 5.8 |
| 41 + d + t | 1 | 0.6 |
| 41 + r | 3 | 1.9 |
| 42 + d | 2 | 1.3 |
| Plants with t and/or a, but without d, tr or r | 34 | 22.1 |

d = dicentric, tr = tricentric, r = ring, t = telocentric, a = acrocentric.

Acro- and telocentric chromosomes were observed in 24% of these plants. Forty-two plants had multicentric or ring chromosomes. The majority of the chromosomal aberrations were observed in plants lacking either one or both 4M$^g$ chromosomes. Two plants had 42 plus one dicentric and one plant had 40 + dicentric + ring + acrocentric chromosome, indicating that chromosome breakage also occurs at a lower frequency in gametophytes with chromosome 4M$^g$.

Plants with multicentric and ring chromosomes underwent chromosome-type BFB cycles as indicated by double-chromatid bridges at mitotic anaphase (Figure 1d,e). Active BFB cycles were observed in 27% of the selfed progeny of a monosomic 4M$^g$ addition plant (Table 2), as indicated by the presence of tricentric, dicentric, and ring chromosomes (Figures 2a–d). The fate of these chromosomes was analyzed in 15 genotypes at different time intervals after germination (Table 3). The majority of BFB cycles ceased about 32 days after germination. Dicentric and ring chromosomes were not observed 84 days after germination, indicating that the broken chromosomes were healed. The longest persistence of a ring chromosome was 32 days, and that of a dicentric chromosome 58 days after planting.

Mitotic metaphases of plants with multicentric and ring chromosomes were analyzed by FISH with centromere- and telomere-specific DNA probes, pRCS1 and pAtT4, respectively. The probe pRCS1 hybridized to all centromeric regions of normal, multicentric, and ring chromosomes (Figure 2a). Multicentric chromosomes had two terminal FISH sites after hybridization with pAtT4. No interstitial pAtT4 FISH sites were observed in these chromosomes (Figure 2b). Ring chromosomes had no telomeric FISH sites with pAtT4 (Figure 2c). Several of the induced telocentric and acrocentric chromosomes lacked detectable telomeric pAtT4 FISH sites at their centromeric ends even when the signal detection was amplified two times (Figure 2b,e).

## Discussion

The data identify chromosome 4M$^g$ as a gametocidal chromosome. The Gc factor on chromosome 4M$^g$, when present in monosomic condition, induces chromosome breakage mainly in gametophytes lacking it. Similar to the Gc factors described previously, the induced chromosome breakage occurs after the meiotic divisions in the interphase prior to the first pollen mitosis (Finch *et al.* 1984, Nasuda *et al.* 1998).

In monosomic 4M$^g$ addition plants, 75% of the gametophytes lack chromosome 4M$^g$ and are prone to chromosome breakage; however, only 29% aberrant ana/telophases were observed at the first pollen mitosis. The level of 4M$^g$-induced chromosome breakage is higher than that induced by the Gc factor located on the *Ae. cylindrica* Host. chromosome 2C$^c$ (9%) but lower than that induced by the strong Gc

*Figure 2.* FISH to metaphase chromosomes of plants derived from a monosomic 4M$^g$ addition plant: (**a**) centromeric probe pRCS1 hybridized to chromosomes of a plant with one dicentric, one tricentric and one telocentric chromosome; (**b**) telomeric probe pAtT4 hybridized to the same metaphase spread as in (**a**); (**c**) centromeric probe pRCS1 hybridized to chromosomes of a plant with one ring chromosome (arrowheads point to the centromeres and arrows to the telomeres); (**d**) telomeric probe pAtT4 hybridized to the same metaphase spread as in (**c**); (**e**) centromeric, a, and telomeric, b to d, signals on telocentric and acrocentric chromosomes observed in different cells of the plant shown in (**a**) and (**b**): note the signal enhancement of the unaltered telomeric sites (arrows) and the lack of telomeric sites (arrow heads) at the broken termini of the telocentric and acrocentric chromosomes. Bars = 10 $\mu$m (**a** and **b** are the same; **c**, **d** and **e** are the same).

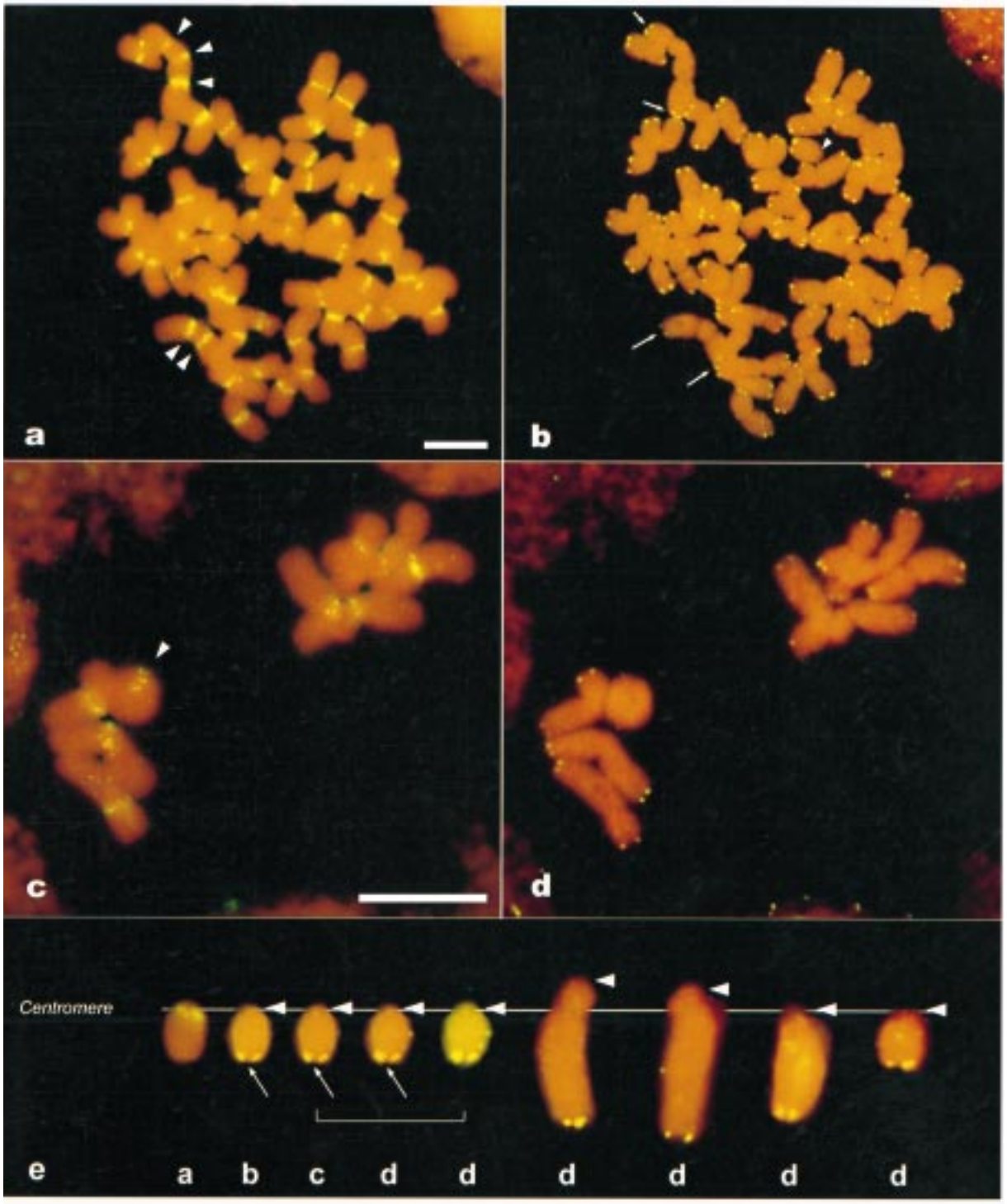
a
b
c
d
Centromere
e
a b c d d d d d d

*Table 3.* Fate of multicentric and ring chromosomes in root tip meristems at different time intervals after germination

| $F_2$ plant | Chromosome constitution | Days after germination | | | | | | Seed set |
|---|---|---|---|---|---|---|---|---|
| | | 3 | 10 | 32 | 58 | 84 | 101 | |
| $F_2$-6-7 | 42 + d | 3 (3) | 3 (3) | 3 (3) | 3 (1) | 3 (0) | 3 (0) | 34 |
| $F_2$-6-12 | 41 + d | 3 (1) | 3 (0) | 3 (0) | 3 (0) | 3 (0) | — | 100 |
| $F_2$-6-14 | 41 + d | 3 (1) | 3 (3) | 3 (0) | 3 (0) | 3 (0) | — | 7 |
| $F_2$-6-15 | 40 + d | 3 (2) | 3 (2) | 3 (0) | 3 (0) | 3 (0) | — | 6 |
| $F_2$-6-16 | 39 + d + d | 3 (1) | 3 (2) | 3 (0) | 3 (0) | 3 (0) | — | |
| $F_2$-6-19 | 41 + r | 3 (3) | 3 (3) | 3 (1) | 3 (0) | 3 (0) | 3 (0) | |
| $F_2$-6-31 | 41 + d | 3 (1) | 3 (1) | 3 (0) | 3 (0) | 3 (0) | — | 9 |
| $F_2$-6-44 | 41 + t + d | 3 (3) | 3 (0) | 3 (1) | 3 (0) | 3 (0) | 3 (0) | 36 |
| $F_2$-6-51 | ? + d | 3 (1) | 3 (0) | 3 (0) | 3 (0) | — | — | 26 |
| $F_2$-6-59 | 41 + d | 3 (2) | 3 (0) | 3 (0) | 3 (0) | — | — | |
| $F_2$-6-60 | 36 + t + r + d + tr | 2 (2) | 2 (2) | 3 (0) | 3 (0) | — | — | 1 |
| $F_2$-6-61 | 40 + a + d + r | 3 (2) | 3 (1) | 3 (0) | 3 (0) | — | — | |
| $F_2$-6-66 | 41 + d | 3 (2) | 3 (0) | 3 (0) | 3 (0) | 3 (0) | — | 2 |
| $F_2$-6-67 | ? + d | 3 (2) | 3 (1) | 3 (0) | 3 (0) | 3 (0) | — | |
| $F_2$-6-73 | 40 + d | 1 (1) | — | — | 3 (1) | — | 2 (0) | |

Harvested (observed) numbers of root meristems showing cells with d and/or r.
— = no root meristems harvested.
d = dicentric, tr = tricentric, r = ring, t = telocentric, a = acrocentric chromosome.

factors *Gc1a* (45%) located on the wheat–*Ae. speltoides* Tausch translocation chromosome T2B-2S and *Gc2* (52%) located on the wheat–*Ae. sharonensis* Eig translocation chromosome T4B-4S$^{sh}$ (Nasuda *et al.* 1998).

Gc factors were previously identified in S-genome species belonging to the section *Sitopsis* and in the C genome of *Ae. caudata* and in the C$^t$ and C$^c$ genomes of the derived polyploids *Ae. triuncialis* L. and *Ae. cylindrica* (Endo 1990). To date, all identified Gc factors were located on homoeologous group 2, 3, and 4 chromosomes and were mapped to their long arms (Endo 1990). This is the first report of a Gc factor present on a group-4 chromosome of the M$^g$ genome of *Ae. geniculata*. Whether similar Gc factors also are present in the diploid M-genome progenitor species *Ae. comosa* Sm. in Sibth. & Sm. var. *comosa* and in the related UM-genome polyploids *Ae. biuncialis* Vis., *Ae. columnaris* Zhuk., and *Ae. neglecta* Reg. ex Bertol. is presently unknown.

The chromosome breakage induced by 4M$^g$ led to the formation of multicentric and ring chromosomes. These chromosomes initiated BFB cycles that can persist in the derived sporophytes. In some plants, the BFB cycles lasted up to 58 days after germination, but ceased about 4 weeks after germination in the majority of plants analyzed. 4M$^g$-induced multicentric and ring chromosomes can be stabilized as either translocation chromosomes or deficiencies and telocentric chromosomes. The lack of cytogenetically detectable telomeric repeats at the broken termini suggests that the broken ends are either not healed or that the number of added telomeric repeats is below the sensitivity of the FISH technique. Recovery of morphologically similar acrocentric and telocentric chromosomes in different cells of plants with active BFB cycles favors the second hypothesis because unhealed truncated chromosomes are unlikely to be transmitted through mitotic divisions (Figure 2e). Werner *et al.* (1992) and Gill & Friebe (1998) reported that first-generation deficiencies induced by the Gc factor located on chromosome 2C$^c$ had telomeric repeats at their broken termini detectable by ISH and FISH analyses. Similarly, telocentric chromosomes derived from bibrancial chromosomes have telomeric repeats attached to originally centromeric termini (Werner *et al.* 1992, Wang *et al.* 1993). Tsujimoto (1993), using the same Gc system, reported that first-generation deficiencies were either lacking or had fewer telomeric repeats at the broken ends, implying that the process of chromosome healing occurs gradually and that the broken termini have

to pass through several cell cycles to acquire the whole repeat length. Our data are in support of a gradual process of chromosome healing.

First-generation deficiencies induced by Gc factors during gametogenesis have already passed through several mitotic cell cycles before being detected in root tip meristems of derived sporophytes whereas chromosomal aberrations produced by BFB cycles either were produced in the preceding mitotic ana/telophase or may have passed through only a few cell cycles. FISH analysis with the telomeric clone pAtT4 on identified chromosomal rearrangements produced by BFB cycles in root tip meristems, at meiotic metaphase I, and in derived progenies will provide unequivocal evidence if chromosome healing by telomere addition occurs gradually or over several cell cycles.

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