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Chapter

Maize Chromosome Abnormalities and Breakage-Fusion-Bridge Cycles in Callus Cultures

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Abstract

The maize karyotype was first characterized by the observation of pachytene chromosomes. The somatic chromosomes were identified by C-banding and FISH with repetitive DNA sequences. C-banding was useful for the identification of chromosome abnormalities in callus cultures. In the present review, we focus on the involvement of heterochromatic knobs on the occurrence of chromosome abnormalities in callus cultures. In a previous work we detected anaphase bridges resulting from delayed chromatid separation at knob regions and typical bridges derived from dicentric chromatids in cultures. The analysis of altered chromosomes showed they were derived from a chromatid-type breakage-fusion-bridge (BFB) cycle. Fluorescent in situ hybridization (FISH) showed signals of telomere sequences in the broken chromosome arm, thus giving evidence of de novo telomere formation on the broken chromosome end. Further observations of long- and short-term cultures have shown the presence of chromosome alterations derived from BFB cycles followed by chromosome healing. Additionally, the occurrence of unequal crossing over in a knob region was observed in callus culture. These results are of interest for studies on the mechanisms of chromosome alterations during evolution.

Keywords: maize, heterochromatic knobs, chromosomal rearrangements, callus culture, breakage-fusion-bridge cycle, unequal crossing over

1. Introduction

Maize is an important crop plant and model organism. The maize karyotype was first characterized by the observation of pachytene chromosomes obtained from pollen mother cells, since the pioneering work by McClintock [1]. The early cytological maps were constructed based on the identification of chromosome relative lengths, arm ratios, heterochromatin patterns, prominent chromomeres, and nuclear organizer region [2–5]. Structures containing heterochromatin were described: heterochromatic knobs, centromeric heterochromatin, B chromosomes, abnormal chromosome 10, and nucleolus organizer region localized on chromosome 6 [6]. Chromosome abnormalities were detected in several investigations, and collections were organized containing reciprocal translocations (A-A translocations), B-A translocations (interchanges between B chromosome and arms of the A set), inversions, and trisomics, available at the Maize Genetics Cooperation

Stock Center [7] (www.maizegdb.org). These materials have been important tools for gene mapping.

The somatic chromosomes were identified by the C-banding procedure which was useful for the identification of chromosomal abnormalities in callus cultures [8–10]. The unequivocal identification of the somatic chromosomes is difficult due to their degree of condensation, and the use of the C-banding procedure was supplemented by an analysis of pachytene chromosomes of the lines from which callus cultures were derived. C-bands correspond to knobs visualized on meiotic chromosomes [11].

The characterization of meiotic and somatic chromosomes was improved by fluorescence in situ hybridization (FISH) using as probes repeated DNA sequences and genes, thus allowing the study of the molecular structure of chromosome components, such as centromere, neocentromere, B chromosome heterochromatic knobs, and gene mapping [12–20].

The maize chromosome structure has been extensively reviewed [21–25]. In the present review, we focus on the involvement of heterochromatic knobs on the occurrence of chromosome abnormalities in maize callus cultures. The size and number of knobs are variable, and they may be present in each of the 10 chromosomes of the complement at fixed positions in modern maize and its relatives, including species of *Zea* (teosintes) and *Tripsacum* [26, 27]. Knobs were mapped on the meiotic chromosomes [2, 3, 5], and recently they were mapped relative to the maize reference genome assembly [19]. Knobs are composed primarily of two tandemly repeated sequences, the 180-bp knob repeat and the 350-bp TR-1 element or a mixture of both [13, 17, 19]. Knobs also contain retrotransposons [28, 29].

One genetic effect attributed to knobs is their influence on recombination [6, 19], and it was revealed that knobs in heterozygous condition can reduce local recombination [19]. Another interesting genetic effect of knobs is their activity as neocentromeres resulting in meiotic drive. This meiotic event is a mechanism by which regions of the genome are preferentially transmitted to the progeny. In maize, meiotic drive is due to an uncommon form of chromosome 10, the abnormal chromosome 10 (Ab 10). In the presence of this chromosome, the knobs of other chromosomes are converted into motile neocentromeres. Thus the knobbed chromosomes preferentially segregate during female meiosis [30, 31]. The origin of maize polymorphism, including size and number, has been discussed in several reports, and it was proposed that meiotic drive was responsible for the evolution of knobs [32]. Recently, a cluster of eight genes on Ab10 was identified, called *Kinesin driver* (*Kindr*) complex, which are required for both neocentromere motility and preferential transmission. It was revealed that Kindr is a strong minus-end-directed motor that interacts specifically with neocentromeres containing 180-bp knob repeats [33].

The effect of knobs on chromosome break and origin of abnormalities in maize callus culture is presented in this review.

2. Chromosome abnormalities in maize callus culture

2.1 Heterochromatin involvement in chromosome breakage

Callus culture is an important step for genetic transformation in plants. The identification of maize genotypes showing high ability to form embryogenic callus type II (friable) and regenerate plants has progressed since the report by Green and Phillips [34]. The genotypes identified since then were adapted to temperate regions [35], and maize genotypes of tropical and subtropical origin have also been shown to produce friable type II calli capable to develop somatic embryogenesis [36–38].

Various studies have shown the occurrence of cytogenetic and genetic variability in plants regenerated from maize callus cultures [39, 40]. This so-called somaclonal variation [41] is undesirable when genetic stability is required, but interesting for the study of mechanisms that give rise to chromosome abnormalities. Chromosome breakage associated with heterochromatin was shown in several plant callus cultures [8–10, 42–48].

Breakpoints involved in chromosome abnormalities associated with heterochromatin were previously detected in maize regenerated plants. The analysis of pachytene chromosomes of these plants revealed that most breakpoints were localized in chromosomes bearing a knob. The authors hypothesized that late-replicating heterochromatin would replicate later in tissue culture, giving origin to bridges in anaphases and occurrence of breakage between the knob and centromere [47]. This would explain the presence of knobs in chromosomes involved in abnormalities observed in regenerated plants. The authors identified in meiotic cells alterations in chromosome structure, such as translocations, intercalary deficiencies, and heteromorphic pairs in 91 of 189 plants regenerated from callus cultures originated from an Oh43-A188 genetic background [47].

2.2 Breakage-fusion-bridge cycle in callus culture and de novo telomere formation

The first reports on breakage-fusion-bridge (BFB) cycles were made by McClintock [49, 50]. In investigations on the behavior in successive nuclear divisions of a chromosome broken at meiosis, it was shown that the chromatid type of BFB cycle initiated by broken chromosome ends occurs in gametophyte mitoses and in the endosperm. In the zygote the broken chromosome ends heal. BFB cycles have also been observed in other species including wheat (*Triticum aestivum*), in which reverse tandem duplications were observed [51]. The chromatid-type BFB cycle initiated in meiosis continued through pollen mitoses and in early endosperm divisions, but did not continue in embryo mitoses.

Investigations of mitotic cells in maize callus cultures detected anaphase bridges resulting in delayed separation at knob regions and typical bridges originated from dicentric chromatids. The observation of C-banded anaphases showed that the chromatids were held together at C-band sites (corresponding to knob) [8]. Typical bridges with and without C-bands were observed. These events were interpreted as derived from a chromatid type of BFB cycle initiated by chromatids that were broken during the primary event.

The analysis of abnormalities in chromosomes 7 and 9 of maize callus cultures gave evidence of their origin from BFB cycles [8–10]. As illustration, we show here the mechanism that would have originated these abnormalities. The callus culture was induced from a hybrid between two sister inbred lines derived from a tropical maize variety (Jac Duro [JD]). These JD lines possessed the same knob composition: K6 L2, K6 L3, K7S, K7L, K8 L1, K8 L2, and K9S [8, 20]. K refers to knob, the number identifies the chromosome, and S refers to short arm and L to long arm. Numbers 1, 2, and 3 refers to knob positions, according to the literature [27]. Thus, chromosome 7 possessed large knobs on both arms, and chromosome 9 had a very large knob on the short arm. Therefore, these chromosomes were more prone to suffer alterations.

An abnormal chromosome 7 carrying two knobs on the short arm was observed in metaphases of a callus culture designed 3–57. This abnormality was interpreted as being a deficiency-duplication (Df-Dp) derived from a BFB cycle and healing of the broken arm, for it was observed in various cells of the culture [8]. **Figure 1** [9, 25] shows the mechanism that would have originated this aberration. The two knobs on the short arm would bear a deficiency in the terminal region (RTD). Therefore this

abnormal chromosome 7 possessed reverse tandem duplications of these knobs and of a segment designated "b."

This chromosome 7 carrying a deficiency on K7S and duplications of the knob and of a "b" segment (Df-Dp7) was stable in culture and was transmitted to regenerated plants. Thus, R_0 plants regenerated from the 3–57 culture were heterozygous for this chromosome alteration. R_1 and R_2 plants were recovered and analyzed. Homozygotes for normal chromosome 7 and heterozygotes for the Df-Dp7 were detected. Plants homozygous for the Df-Dp7 were not recovered. Presumably, seeds carrying homozygotes were not viable. **Figure 2A** [9, 25] shows a metaphase of a
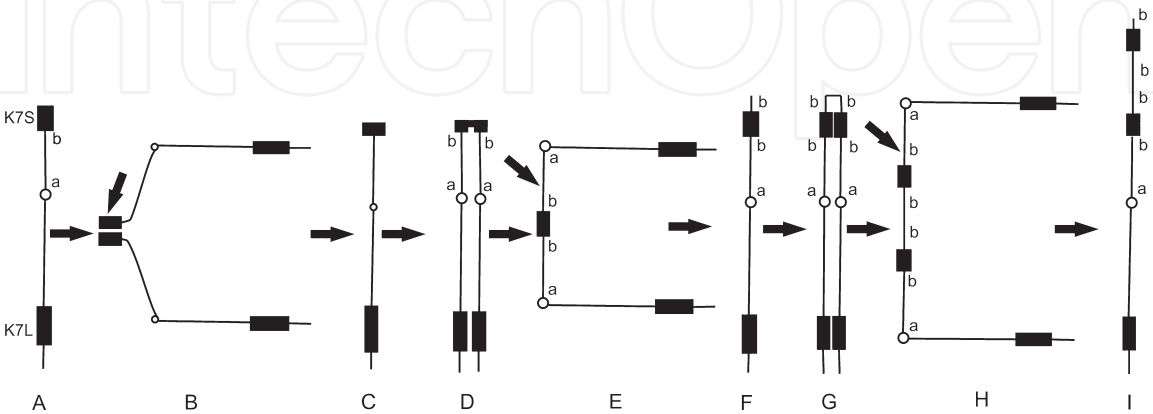
Figure 1.
BFB cycle that would give rise to chromosome 7 bearing a deficiency and duplication (Df-Dp) showing normal chromosome (A); anaphase with delayed separating chromatids and breakage at K7S (B); chromatid with a deficient K7S (C), fused after replication (D); breakage at anaphase (E); chromatid with duplication of the "b" segment (F), fused at broken ends (G); anaphase bridge and breakage (H); resulting chromatid with two knobs and reverse tandem duplications (RTD) of the "b" segment. Arrows at anaphases indicate breakpoints [9, 25].
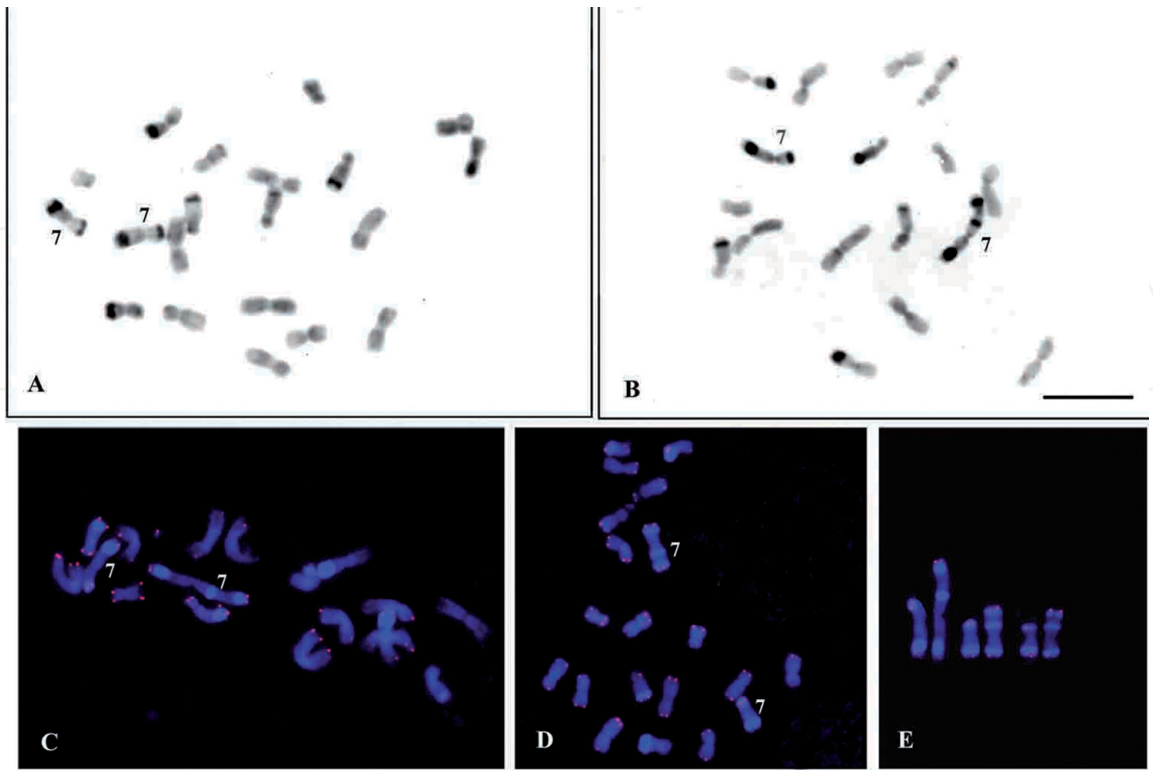
Figure 2.
Somatic chromosomes of R_1 plants derived from the 3–57 culture. C-banded mitotic metaphases homozygous for normal chromosome 7 showing knobs (C-bands) on the long and short arms (A); heterozygote for the Df-Dp chromosome 7 with two knobs; telomeric FISH signals on early and full metaphases (C, D); homologous pairs of chromosome 7 [9, 25].

regenerated plant homozygous for normal chromosome 7, and **Figure 2B** shows a metaphase of a plant heterozygous for the aberration. The distal knob (K7S) is subterminal, for there is a tiny terminal euchromatic segment on the short arm.

Fluorescent in situ hybridization (FISH) using the telomeric sequence $(TTTAGGG)_6$ showed signals in all the somatic chromosomes of the regenerated plants, including the Df-Dp7 chromosome (**Figure 2C–E**) [9, 25]. This result gives evidence of telomere healing at the end of the broken short arm. In these DAPI-stained metaphases, bands corresponding to the knobs could be clearly visualized. In the less condensed metaphases, the telomeric signals could be detected at the end of the euchromatic segment of the duplicated short arm.

The healing of chromosome ends, i.e., the addition of telomere sequences to the broken chromosome ends, has been observed in diverse plant species. In wheat, FISH telomeric signals were detected at the broken ends of deleted chromosomes and at the centromeric regions of telocentric chromosomes [52, 53]. The expression of telomerase has been reported for diverse plant tissues, such as the meristematic tissue and suspension cell cultures [54]. In barley, there was a decrease in the number of telomeric sequences in differentiated cells, and the number of telomeric sequences increased in callus cultures [55]. High telomerase activity was observed in calli derived from tobacco plants, while in leaves the activity was very low [56]. In wheat, during the divisions of the gametophyte, dicentric chromosomes undergo BFB cycles. De novo addition of telomere sequences occurs gradually during the early mitotic divisions in the sporophyte [57].

The present study showing the telomere healing of the broken short arm of chromosome 7 gives evidence of telomerase expression in maize callus culture. The addition of telomeric repeats occurred on a euchromatic region, which was certainly non-telomeric.

The meiosis of the regenerated plants heterozygous for the Df-Dp chromosome 7 was normal. The terminal euchromatic segment was clearly observed at pachytene stage on the duplicated short arm. In the diplotene and diakinesis stages, a heteromorphic pair corresponding to chromosome 7 was observed, as expected for heterozygotes bearing a duplication [9].

2.3 Chromosome 7 and 9 abnormalities in long-term subcultures

C-banded metaphases of subcultures prepared after 18 months of the initiation of the 3–57 callus culture were analyzed during a cultivation period from the 18-month-old original culture to 42-month-old cell lines. The subcultures were designated as cell lines 1-MS2, 2-MS-2, 1-MS1, 2-MS1, 1 N6, and 2 N6. Feulgen-stained anaphases were also observed.

The investigation of mitotic instability by the analysis of Feulgen-stained anaphases showed abnormalities similar to those previously described [8] and shown in **Figure 3**: (i) bridges resulting from delayed chromatids held together at knob sites (**Figure 3A**), (ii) broken bridges (**Figure 3B**), (iii) typical bridges (**Figure 3C, D**), and (iv) fragments (not shown) [9]. The analysis of the frequency of these abnormalities showed a tendency of decreasing frequency with time in culture. Three samples of each cell line were harvested in different periods of cultivation, except 1-MS1 from which seven samples were analyzed. The frequency of anaphase abnormalities observed varied from 4 to 10% in the first sample and from 0.67 to 5.33% in the last sample. This tendency of decreasing frequency was a consequence of the healing of the broken chromosomes, therefore, avoiding an accumulation of BFB cycles [9], as discussed below. Interestingly, the total frequency of abnormalities varied from 0.67 to 10%, and this result was quite similar to the ones observed in a previous study of 5-month-old cultures derived from related inbred lines [38].
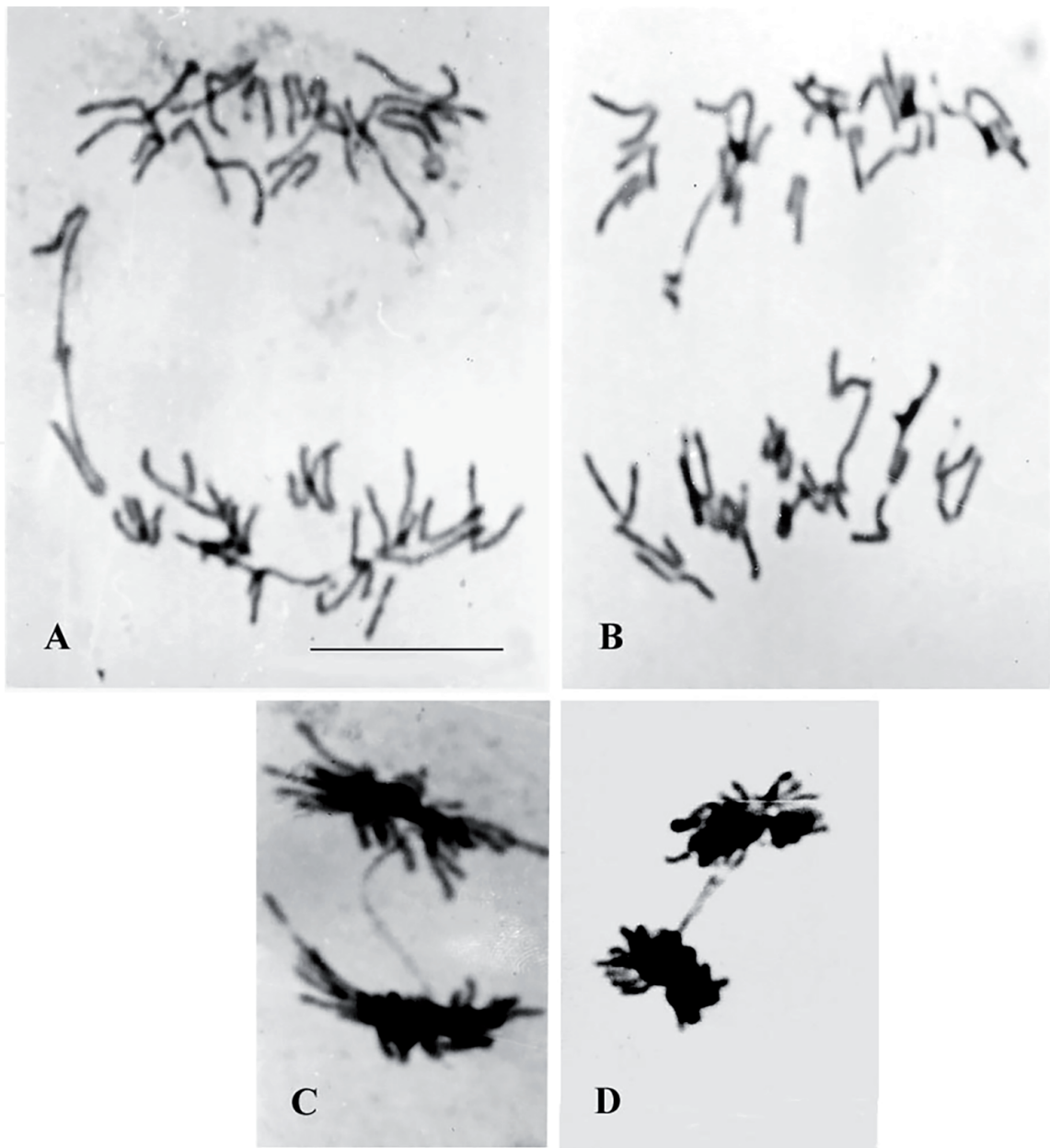
Figure 3.
Feulgen-stained anaphase cells of the cell lines derived from the culture 3–57, with a laggard chromosome showing delayed separating chromatids (A), broken bridge (after the primary event) (B), typical bridge (C), and double bridge (D). Scale bar = 10 μm [9].

The analysis of the cell line pedigree showing the types of chromosomes 7 and 9 observed in C-banded metaphases in different subcultures of the six cell lines is displayed in **Figure 4** [9, 25]. A karyotype diversity among cell lines was detected in this analysis, but homogeneity within some of them was observed in samples harvested at different age transfers. Then, new abnormal chromosomes were stable in different subcultures. Gross aberrations were not observed in chromosomes 6 and 8 that possess knobs smaller than those found in chromosomes 7 and 9.

Different types of abnormal chromosomes 7 and 9 were observed in the cell lines. In the original 18-month-old callus culture 3–57, two types of chromosome 7 were detected. One of the chromosomes possessed two knobs on the short arm (K7S) corresponding to the Df-Dp chromosome 7 described above, and the other type possessed K7S on an interstitial position of a duplicated short arm (**Figure 4**). This chromosome would have originated from a mechanism similar to that shown in
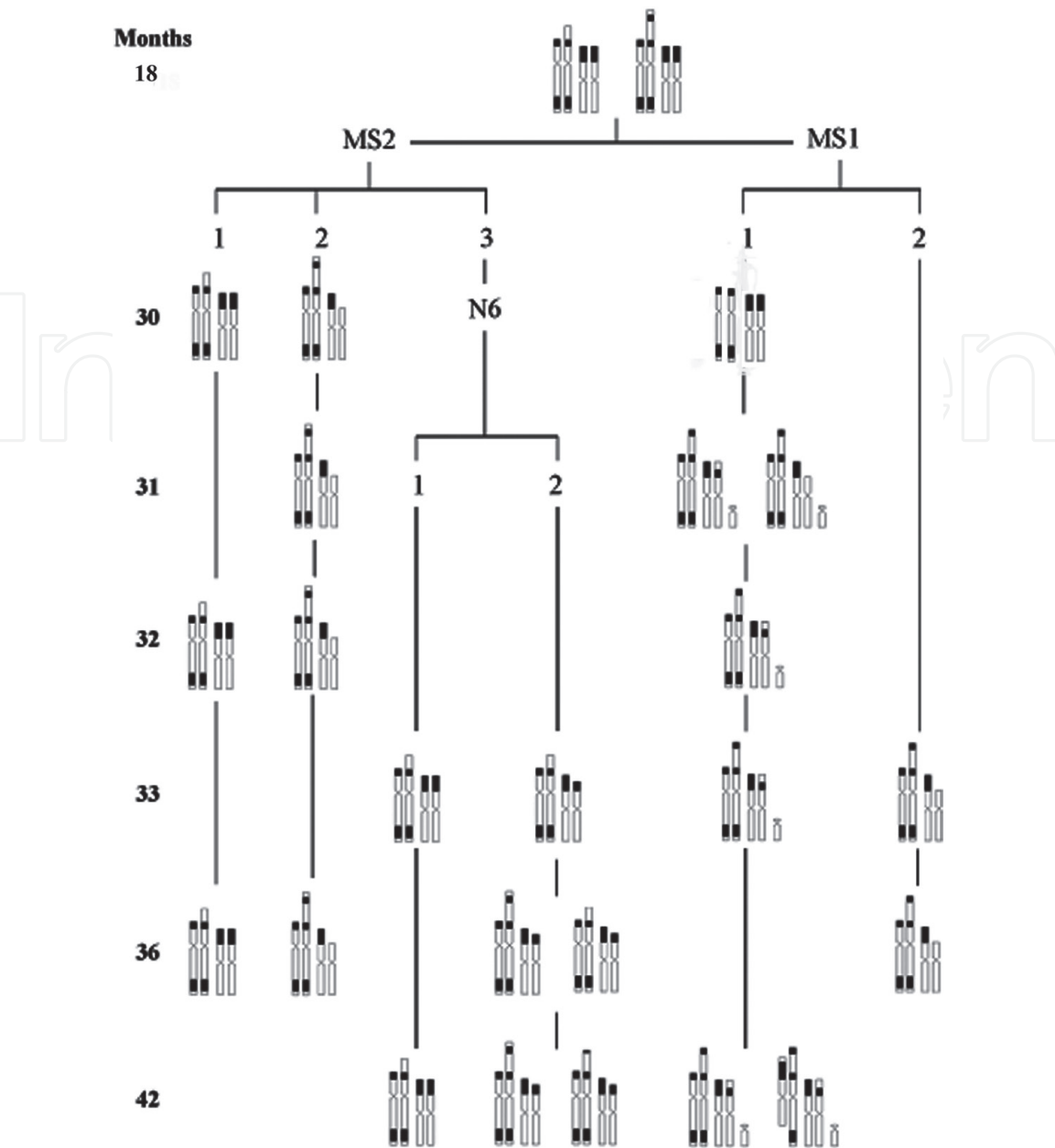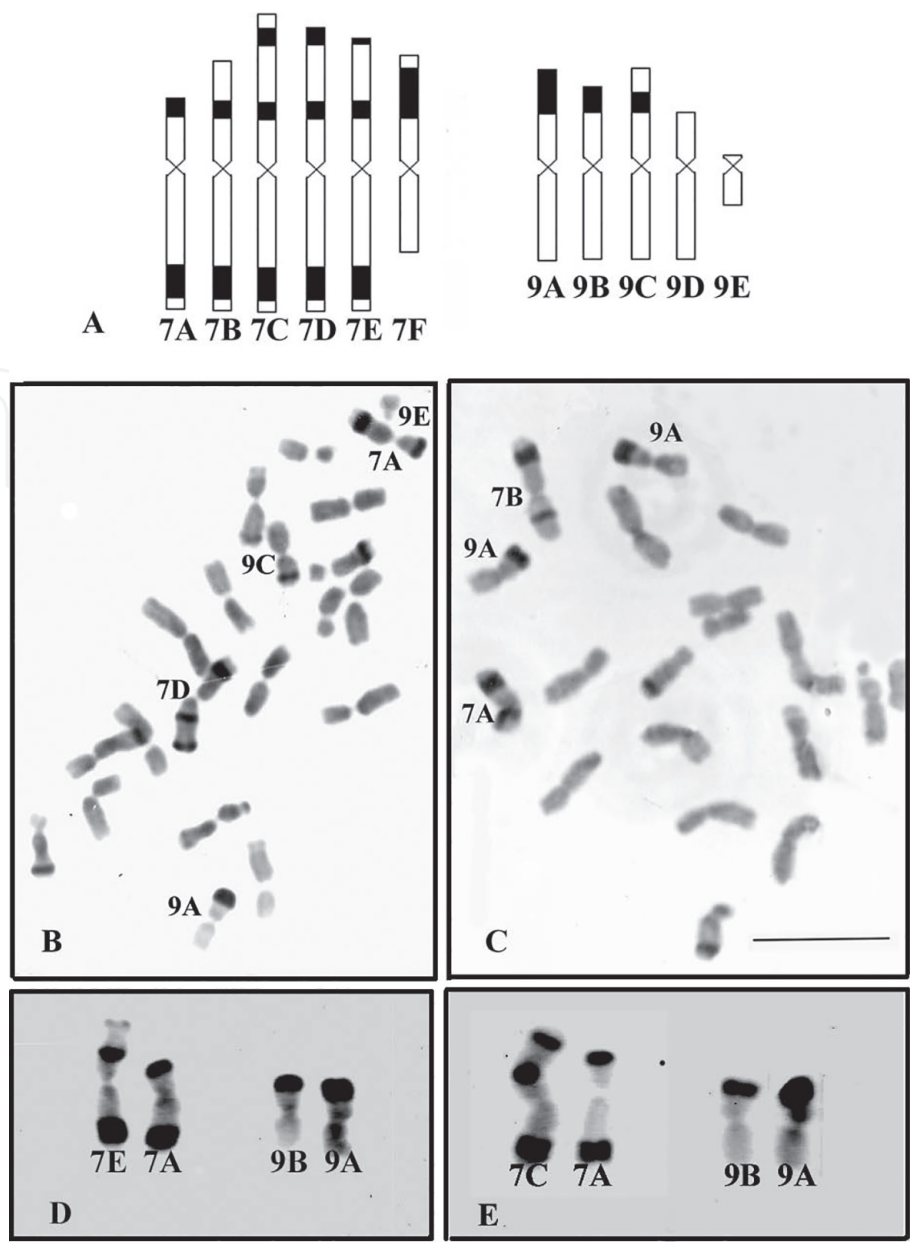
Figure 4.
Cell line pedigree showing the types of chromosomes 7 and 9 observed in C-banded metaphases in different subcultures of the six cell lines derived from culture 3–57 [9, 25].

Figure 1 [9, 25]. The following types of chromosome 7 were distinguished in the cell lines (**Figure 5A**): 7A, normal type, with a terminal K7S and a subterminal K7L; 7B, with a duplicated short arm and a subterminal K7S; 7C, with two knobs on the short arm and a terminal euchromatic segment (similar to **Figure 2B**); 7D, similar to 7C, but without the terminal euchromatic segment; 7E, similar to 7D, with a smaller deficient and terminal K7S; 7F, with a larger short arm, a very large interstitial K7S and without the K7L on the long arm. The 7A, 7B, and 7C types were found in the original 18-month-old culture (**Figure 4**). **Figure 5B**, **C** illustrates the 7D and 7B types, respectively, and the 7E type can be seen in **Figure 5D**. **Figure 5E** illustrates the 7C chromosome. The 7F type can be seen in the pedigree of the 1-MS1 cell line (42-month-old culture, **Figure 6**) [9].

Different types of altered chromosome 9 were also observed in the samples of cell lines (**Figure 5A**) [9]: the normal type corresponds to 9A; a smaller terminal K9S corresponds to 9B; a smaller subterminal K9S corresponds to 9C; 9D is a chromosome without the knob; and 9E is a minichromosome derived from chromosome 9.
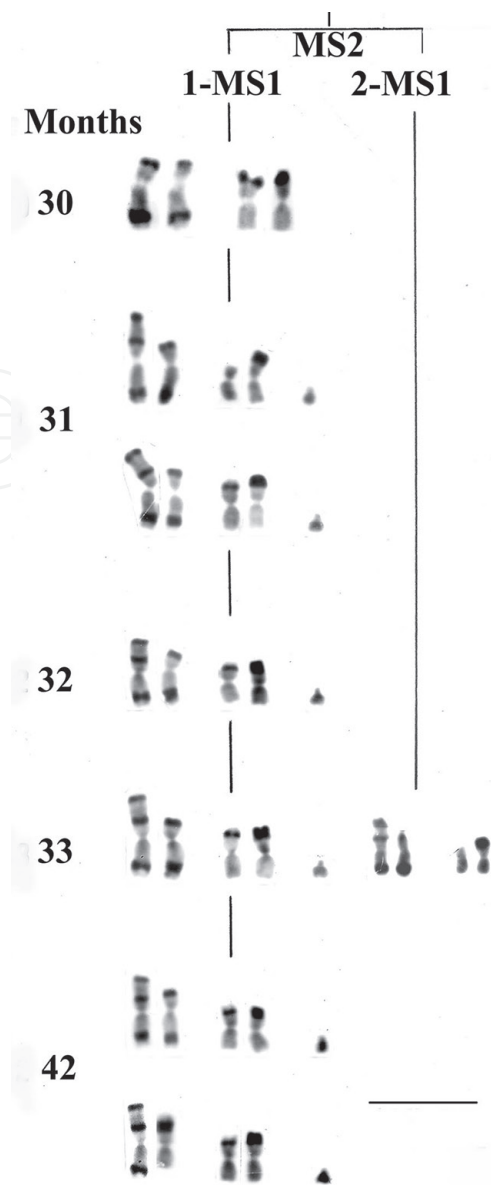
Figure 5.
Types of chromosomes 7 and 9 observed in C-banded metaphase of the cell lines from culture 3–57 (A); metaphase cells of the 1-MS1 cell line (B) and 1MS2 (C); chromosomes 7 and 9 of the N6 cell line (D, E). Scale bar = 10 μm [9, 25].

The 9A, 9C, and 9E types can be seen in **Figure 5B**, and the 9B type is shown in **Figure 5D**, **E**. **Figure 6** shows the 9D type, which appears in the 31-month-old subculture of the 1-MS1 cell line. This figure illustrates the different types of chromosomes 7 and 9 detected in 1-MS1 cell line [9, 25].

Therefore, the analysis of metaphases of the cell lines showed new abnormalities in chromosomes 7 and 9. The occurrence of delayed chromatid separation and bridges in anaphases provided evidence of BFB cycle events, and healing of the broken chromosomes could be inferred by the stability of the same abnormal chromosome in different subcultures of the same cell line [9, 25].

In most cell lines, the original abnormal chromosomes 7 (7B and 9C types) were maintained. The 7E type (with a smaller distal K7S) was found in the 42-month-old subculture of the 2-N6 cell line (see **Figure 4**) [9, 25]. The 7D type (chromosome 7 without the terminal euchromatic segment) was observed in the 1-MS1 and 2-MS1. These data suggest that cells bearing the original Df-Dp chromosome 7 (7B or 7C types) were highly adapted in culture and that the new types (7D and 7E) found in
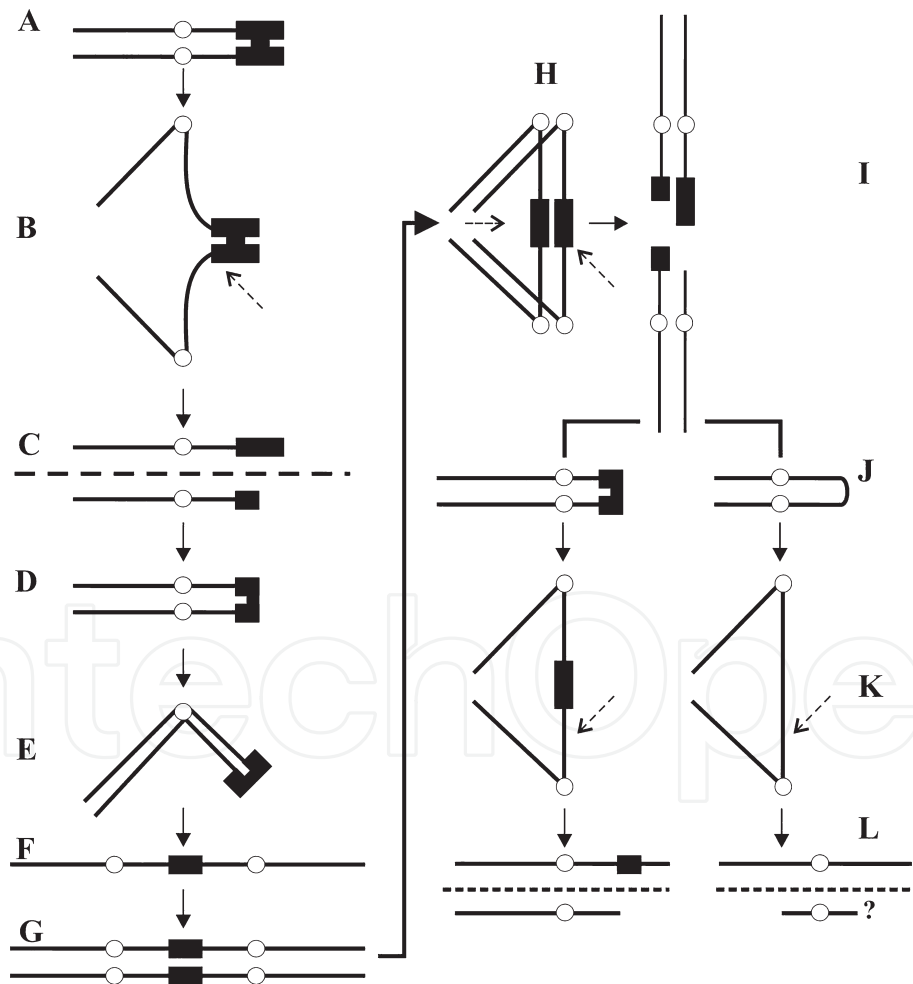
Figure 6.
Types of chromosomes 7 and 9 observed in C-banded metaphases of the 1-MS1 and 2-MS1 cell lines. Scale bar = 10 μm [9].

some subcultures were derived from the original altered chromosome 7 (7C type) through new events of delayed chromatid separation at the knob region and breakage. The 7F type would be a new alteration of the normal chromosome and was detected in the 42-month-old subculture of the 1-MS1 cell line (**Figures 4, 6**) [9, 25]. Its origin would be through a delay in sister chromatids on K7S at anaphase, and an amplified subterminal knob would appear if the duplicate knob did not separate and a breakage occurred at an adjacent euchromatic region. A delayed separation of chromatids on K7L at anaphase, followed by breakage, would explain the absence of this knob in the 7F type.

Chromosome 9 suffered alterations in most cell lines, except for the 1-MS2 and 1-N6 cell lines. The 9D type (K9S deleted) was detected in the 2-MS2 and 2-MS1 cell lines, and the 9B type (partial deletion of the knob) was observed in the 2-N6 cell line (see **Figure 4** [9, 25]). A total or partial deletion of K9S would have occurred after a delay of separation of the chromatids on this knob region and breakage totally or partially eliminating the knob or a segment of it. Interestingly, in the cell line 1-MS1, two types of chromosome 9 appeared, the 9C displaying a subterminal smaller K9S (9C type) and a chromosome without the knob (9D type).

In addition, a minichromosome (9E type) appeared in the subcultures possessing one of these abnormal types. These abnormalities could have resulted from the mechanism suggested in **Figure** 7 [9, 25]. The primary event would be a delay in the separation of chromatids at K9S region followed by breakage originating a deficient knob. Then, two types of BFB cycles, the chromosome [58] and the chromatid types, would have originated the 9C, 9D, and 9E chromosome types. The 9C and 9E chromosome types were observed in several subcultures, thus providing evidence of healing of the broken chromosome ends. In the cell lines analyzed, abnormalities were detected only in chromosomes 7 and 9. These alterations were derived from a primary event of chromatid delayed separation at knob sites in anaphases, followed by breakage and BFB cycle. The presence of large knobs in these chromosomes would lead to this kind of primary event. A case of elimination of chromosome segments from knobbed chromosomes was reported by Rhoades and Dempsey [59]. In the presence of B chromosomes, a bridge formation would occur due to delayed replication of the knob at the second microspore division. Chromosomes containing large knobs would be involved more frequently in this kind of event.

Figure 7.
Mechanism that would originate different types of chromosome 9 in the 1-MS1 cell line: Normal chromosome (A); anaphase with delayed separating chromatids and breakage (B); the resulting chromatids, one normal and the other with a deficient knob (C) fused after replication (D); nondisjunction of sister chromatids (E) resulting in a dicentric chromosome (F, G); double bridge (H) giving rise to a chromatid with a deficiency at K9S and another chromatid without knob (I), which after duplication and fusion (J) suffered breakage in the next anaphases (K); resultant chromosomes: One with a deficient K9S, another without the knob, and a minichromosome originated after deletions whose mechanism is unclear (L). Arrows at anaphases indicate breakpoints [9, 25].

2.4 Heterochromatic knob amplification resulting from unequal crossing over and BFB cycle

The observation of mitotic and meiotic aspects of an amplification of the knob localized on the long arm of chromosome 7 (K7L), in plants regenerated from a long-term callus culture designated 12-F, was carried out aiming to investigate the origin of this amplification. The 12-F original culture was 28 months old when the R1 plants were obtained [10]. The original callus 12-F was heterozygous for the amplified K7L. Therefore, segregation was expected in R_1 progenies derived by selfing R_0 plants. Plants homozygous for the normal and amplified K7L, and plants heterozygous for the amplified K7L, were recovered (**Figure 8**) [10]. The frequency of plants homozygous for the amplification was lower than expected according to Mendelian segregation, while the frequency of plants homozygous for the normal K7L was higher than expected. The frequency of heterozygotes was according to the expected value.
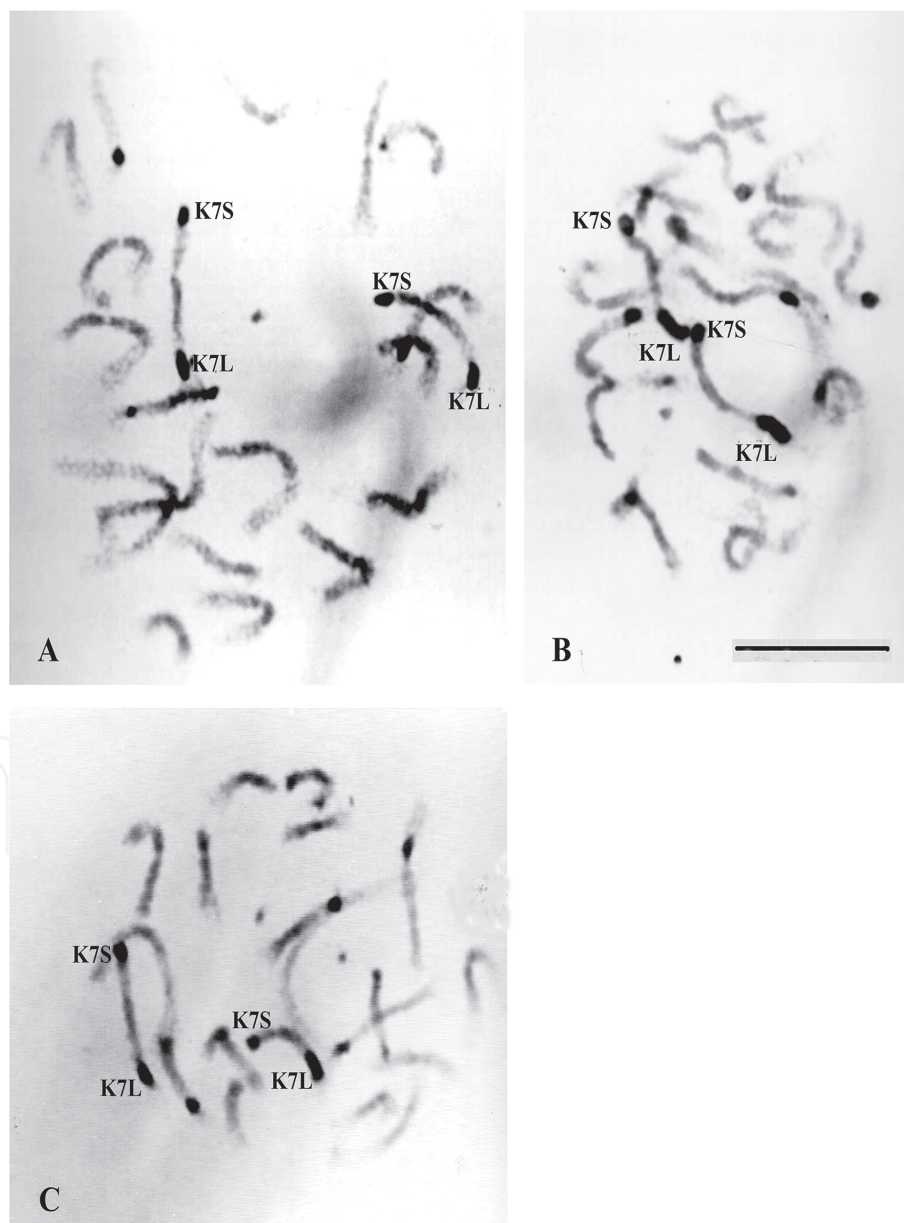
Figure 8.
Somatic chromosomes of regenerated plants (R_1) derived from the 12-F culture: C-banded prometaphase of the homozygote for normal chromosome 7 (A); homozygote for the amplified K7L (B); heterozygote for the amplified K7L (C). Scale bar = 10 μm [10].

Some plants whose karyotype was investigated were selected for meiotic analysis. The homologous chromosomes were completely synapsed on knobs and terminal euchromatic segment on the long arm at pachytene in plants homologous for normal and amplified K7L (**Figure 9A**, **B**) [10]. In plants heterozygous for the amplification, the knobs and the terminal euchromatic segments were completely synapsed in some cells (**Figure 9C**) [10], but synapsis failure was also detected in these regions (**Figure 9D**) [10]. In chromosomes bearing the K7L amplification and in normal chromosomes, the size of the distal euchromatic segment was similar, but the size of the amplified knob was significantly larger than the normal knob. In a possible case of delay of chromatid separation at this knob site followed by breakage and a BFB cycle, the distal euchromatic segment would be lost as discussed below [10].

Other abnormalities such as translocations, inversions, duplications, and deletions were not found in the chromosomes of these plants derived from a long-term callus culture [10].

The analysis of microsporocytes at the diakinesis stage showed the presence of two types of univalents: one larger with two C-bands, thus corresponding to chromosome 7, and a small one. The frequency of univalents was low for both types. The frequency of the large univalents in heterozygous plants was higher (2.88%) than control plants (0.55%). Differences were not observed in the frequency of small univalents in the heterozygotes (1.14%) in comparison with control plants (1.92%) [10]. Therefore, the meiosis was normal in most microsporocytes, and R2 progenies were also obtained.

The investigation of short-term cultures derived from inbred lines and hybrids related to the inbred line donor of culture 12-F showed interesting alterations on the
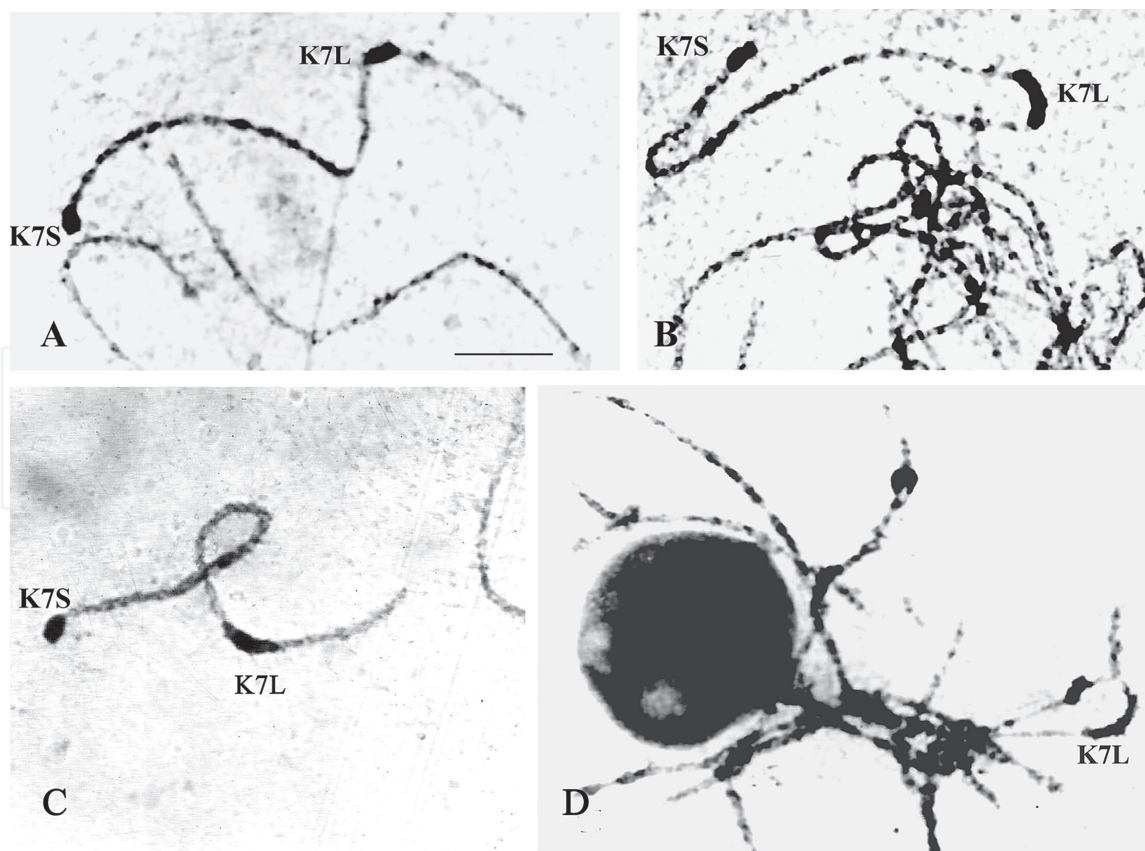
Figure 9.
Carmine-stained meiotic chromosomes of R₁ plants derived from the 12-F culture. Pachytene of a homozygote for the normal K7L (A); homozygote for the amplified K7L (B); heterozygote for the amplified K7L, showing completely synapsed chromosomes (C); pairing failure in the knob region of the heterozygote. Scale bar = 10 μm [10].

long arm of chromosome 7 bearing K7L. The cytogenetic analysis of these cultures detected abnormalities in chromosomes 7 and 9 and other chromosomes with and without knobs. Here we focus only on alterations in the long arm of chromosome 7 to infer the origin of these abnormalities, aiming to understand the origin of the K7L amplification observed in plants derived from culture 12-F. A total of 5223 cells of the callus cultures from 6 genotypes were examined. In three cells from different cultures, chromosome 7 bearing with asymmetric C-bands (corresponding to K7L) was observed: one band was amplified, and the other was reduced in sister chromatids (**Figure 10A, B**). These band alterations would have appeared due to the occurrence of unequal sister chromatid recombination [10].

Unequal crossing over in regions containing duplicate genes or repetitive DNA has been demonstrated in several organisms, such as yeast [60], apes [61], and humans [62]. Two reciprocal products, a directly amplified tandem duplication and a deletion, can result from unequal crossing over between homologous chromosomes. In patients with chromosome duplications involving some types of Charcot–Marie–Tooth disease, the occurrence of unequal recombination between homologous segments (interchromosomal) and sister chromatids (intrachromosomal) has been shown [62]. Thus, the generation of deletions and duplications by unequal recombination can affect the copy number of repeated genes and noncoding repeated DNA sequences.
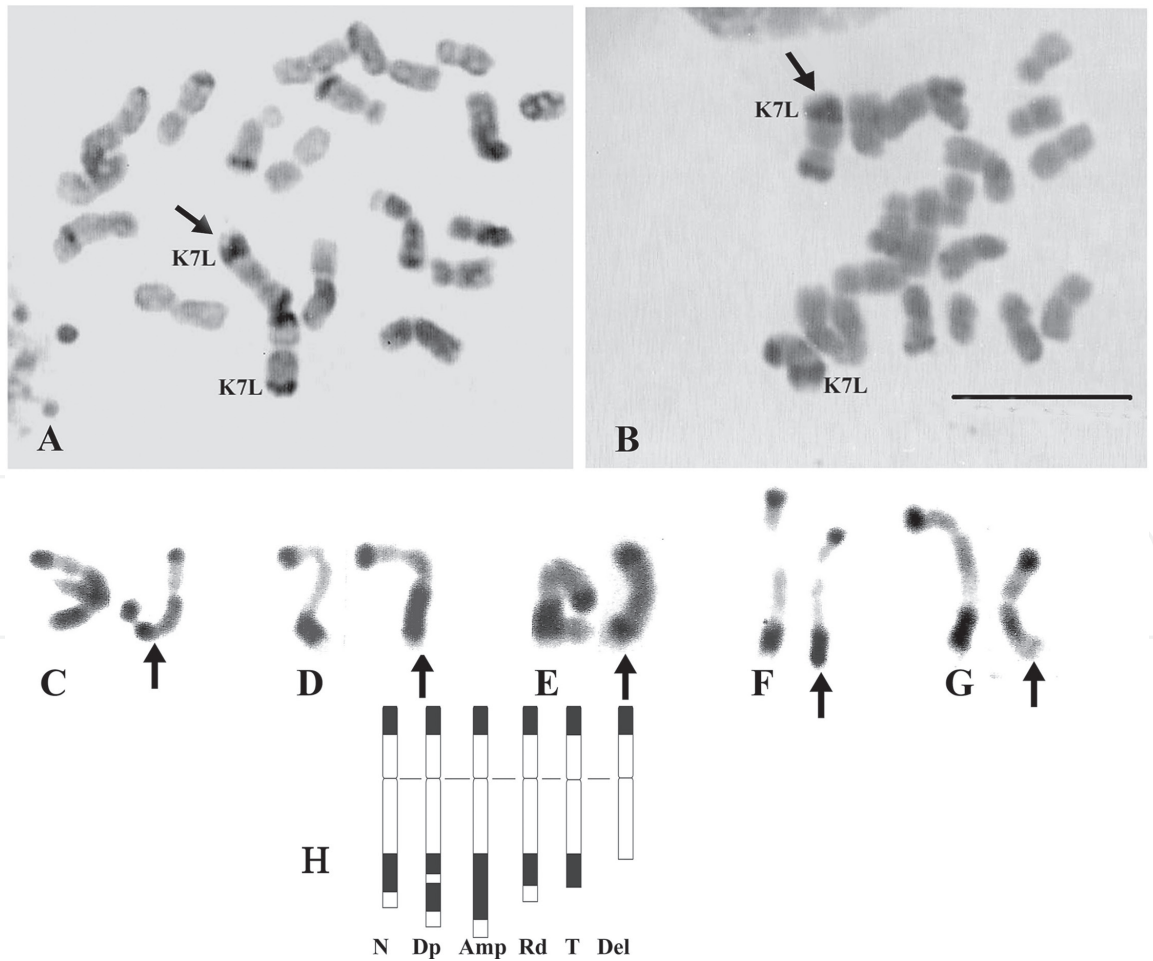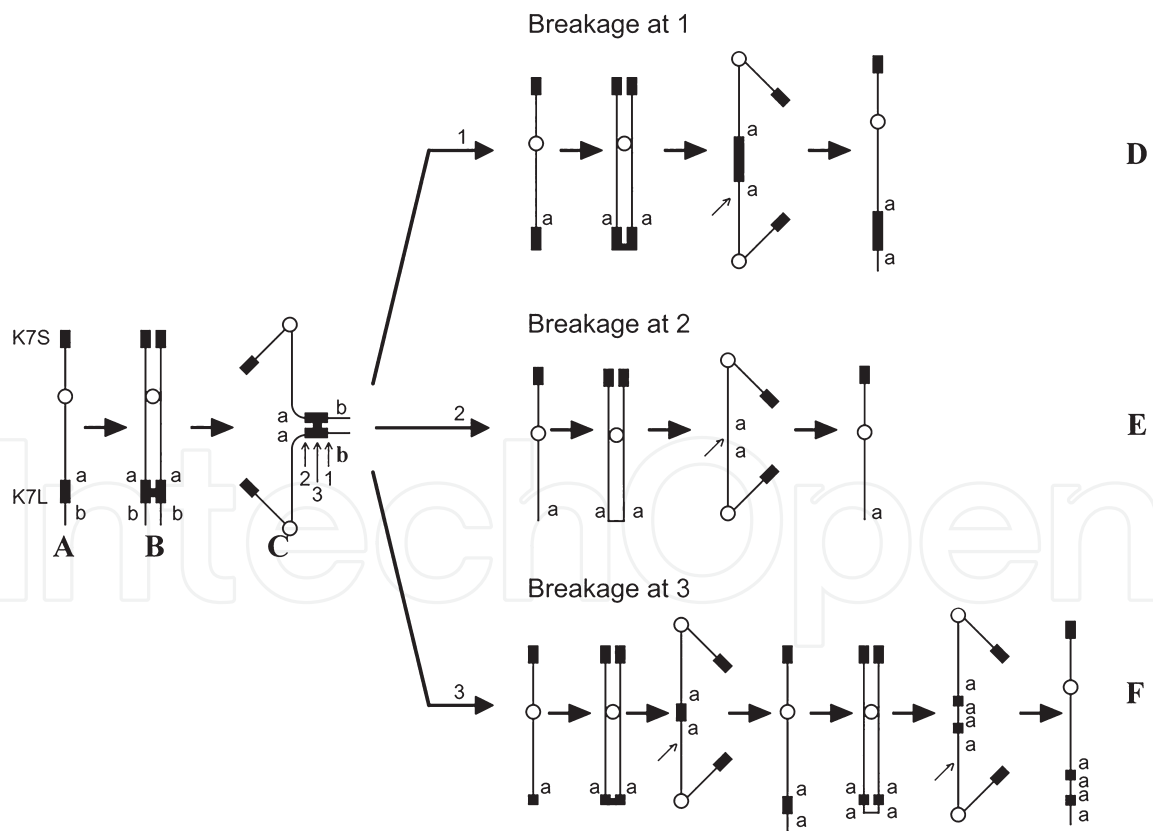

Figure 10.
Types of alterations in the long arm of chromosome 7 observed in C-banded metaphases of the short-term callus cultures: K7L with different sizes in the sister chromatids (A, B); K7L duplication (C); K7L amplification (D); K7L reduction (E); K7L in the telomeric position (F); absence of K7L (G); diagrammatic representation of the abnormal chromosomes (H). Arrows point to the altered chromosomes. N, normal; Dp, duplication; amp, amplification; Rd., reduction; T, terminal; Del, deletion. Scale bar = 10 μm [10].

From this scenario, we can assume that the asymmetric chromosome 7 observed in callus cultures resulted from unequal chromatid recombination at the K7L site. Therefore, the amplification of K7L detected in R1 plants derived from the 12F culture would have originated from an unequal recombination at K7L. This event would not alter the size of the distal euchromatic segment, as observed here [10].

Other alterations were observed on the long arm of chromosome 7 in the callus cultures analyzed: duplication of K7L (**Figure 10C**), amplification of K7L (**Figure 10D**), reduction of K7L (**Figure 10E**), K7L localized on telomeric position (**Figure 10F**), and the absence of K7L (**Figure 10G**). **Figure 10H** shows a diagrammatic representation of these abnormalities. The frequency of these aberrations varied from 0.98 to 4.82% in the six genotypes evaluated [10].

These abnormalities could have originated from a delay of sister chromatid separation at the K7L region, followed by breakage and BFB cycles as suggested in **Figure 11** [10]. After the primary event of delayed chromatid separation, breakage could occur in three different positions at the knob region, terminal (1), proximal (2), and middle (3), giving rise to the different types of abnormalities shown in **Figure 10C–G**. Note that in all possible events, the terminal euchromatic "b" segment would be lost. Regenerated plants homozygous for these aberrations probably would not survive with the deletion of the terminal segment. The recovery of regenerated plants homozygous for the K7L amplification gives support to

Figure 11.
Diagrammatic representation of the BFB cycles that would result in abnormalities in the long arm of chromosome 7 observed in the short-term callus cultures. The normal chromosome (A) duplicates after DNA replication (B) and delayed separation of the chromatids is observed (C). Breakage occurs at three possible positions: Terminal (1), proximal (2), and middle (3). The resulting chromatids are the following: Breakage at position 1 originates a chromatid with a terminal knob or an amplified knob after a new mitotic cycle (D); breakage at position 2 results in chromatids with the absence of the knob (E); breakage at position 3 results in a reduced terminal knob after the primary event, in a reduced knob and a terminal euchromatic segment after a new mitotic cycle or a duplicated knob after a third mitotic cycle (F). "A" and "b" represent segments near the knobs at a proximal and at a distal position, respectively. Note that in all events, the "b" segment is lost [10].

the hypothesis that this amplification was originated from an unequal chromatid amplification.

Therefore, the results show that knob amplification or reduction can appear as a result of BFB cycles or unequal crossing over, but if they are originated from BFB cycles, they would not survive in homozygous regenerated plants.

3. Conclusions

The presence of some chromosome abnormalities in maize callus cultures can be explained by the occurrence of delay of chromatid separation in mitotic anaphases. This primary event gives origin to a bridge followed by a breakage-fusion-bridge cycle and chromosome healing. FISH using telomere sequences as probes gave evidence of de novo telomere formation at broken chromosome ends. Amplifications and deficiencies in the knobs may also occur via unequal chromosome crossing over evidenced in culture by the presence of chromosome 7 showing differences in the size of K7L (C-band) in sister chromatids.

The data suggest interesting questions for further investigations such as the mechanism underlying the delay in chromatid separation at knob sites and that of de novo telomere formation at the broken chromosome ends in callus culture. Changes in DNA methylation could be the cause of unusual later replication of knobs (see [40]).

The observations on the chromosome healing of chromosomes 7 and 9 showed that this event occurred in euchromatic and heterochromatic regions, certainly non-telomeric sites. Mechanistic information on telomere formation is available through studies on *Saccharomyces cerevisiae*. In this species, telomere sequences were added to non-telomeric broken chromosome ends, but a strong preference for telomerase action was observed at GT, TG, or GG nucleotides [63]. In wheat, de novo telomere formation was initiated by 2-to-4 nucleotide target motifs in an rDNA sequence localized in terminal position [53]. Homologous recombination, nonhomologous end joining, and de novo telomere formation are different mechanisms that repair DNA double strand. De novo telomere formation would be a rare event [64]. In the present report, the healing of chromosomes 7 and 9 possibly occurred in the presence of internal sequences to which telomerase was recruited.

In conclusion, mechanisms of chromosomal evolution like the related here might occur in plants. It has been suggested that structural chromosomal rearrangements frequently appear in euchromatin-heterochromatin borders [65].

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Conflict of interest

The authors declare no conflict of interest.

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