Chromosomal instability in immortalized cell cultures of maize

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Abstract

Among the several causes of the genomic variation commonly observed in plant cell cultures, the chromosome instability has been more frequently registered. The objectives of the present research was to accomplish a cytogenetical analysis to verify the mitotic stability in cell cultures of maize, which has been continuously cultivated for more than 14 years. Cellular cultures were induced from immature embryos, subcultured monthly, and the friable regions selected for the maintenance of somatic embryogenesis. Chromosome analysis was performed with fragments of calluses collected from the surface of the embryogenic cultures. The results indicated the maintenance of 6% to 10% of chromosomal abnormalities out of the total of anaphases and telophases analyzed. The observed configurations included: chromosome bridges, chromatid fragments, lagging chromosomes, delay in the separation of sister chromatids and micronuclei at telophases. Although the regeneration ability reduced significantly in comparison to the initial periods of the cultures, the observed chromosomal stability and continuous cell proliferation rate indicated that these cellular lineages have reached a status that may be considered "immortalized". It was observed that the nature and the mechanism of occurrence of the chromosomal abnormalities remained identical to those observed at the beginning of the cultures. These results are highly promising in the direction to make possible the use of these cultures in studies on the cellular aging process and the evaluation of the consequences of the long term cultures in the genome stability.

Introduction

In vitro culture of cells has led to several useful approaches for plant biotechnology, such as: the selection and clonal propagation of promising genotypes and the production of transgenic cultivars, among others. However, for those purposes, it is necessary that the cell cultures maintain their ability to regenerate fertile plants without showing genetic variation, even after long periods of in vitro culture. Among the several causes of the somaclonal variation commonly observed in plants regenerated from cell cultures, the chromosome instability has been more frequently registered. Usually, an increased frequency of genetic and chromosomal changes is observed in plants regenerated from in vitro cultured cells.

In annual plant species, normal somatic cells are able to divide only a limited number of times before they become senescent. During the past decades, the study of animal cell senescence and immortalization has entered the mainstream of cancer research. It has been found that some of the most common genetic changes known to occur in cancer have a key role in the immortalization process. Thus, the ability to produce long term in vitro cultured cell lineages mean that many more cell divisions are required for embryogenesis than it is possible unless cells breach the senescence proliferation barrier and become immortalized.

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According to one hypothetical mechanism (Phillips et al., 1990; Kaeppler and Phillips 1992), changes in DNA methylation could affect the structure of the chromatin, leading to the even later or further delayed replication of heterochromatin. These alterations could be responsible for the formation and breakage of chromosome bridges at mitosis. The initial events responsible for the occurrence of structural changes in chromosomes of cultured cells are characterized by the delayed segregation of sister chromatids in mitotic anaphases, originating preferentially at heterochromatic knobs (Fluminhan and Kameya, 1996; Fluminhan et al., 1996a). Such mechanism also has been observed at the first mitoses of germinating roots from aged seeds (Fluminhan and Kameya 1997; Scandolieri et al. 2006). The role of knob heterochromatin has been discussed elsewhere (Fluminhan et al., 1996b; Aguiar-Perecin et al., 2000) and the extensive occurrence of methylated Cytosine on the maize heterochromatic knobs has been demonstrated previously (Fluminhan et al., 1997).

The objectives of the present research were to accomplish a cytogenetical analysis to verify the mitotic stability in a cell culture of maize maintained for long period, as well as to investigate the occurrence, nature and frequency of chromosomal aberrations in mitotic anaphases of a long term embryogenic callus culture that were induced from a tropical maize synthetic cultivar.

Material and Methods

Cell cultures were induced from immature embryos removed from a synthetic cultivar which was obtained by the intercrossing of 12 commercial hybrids. Friable regions were selected for the maintenance of somatic embryogenesis and the growing calluses were subcultured monthly.

In vitro performance of the cultivar was evaluated following standard protocols based on the report from Green and Phillips (1975) and the embryogenic callus cultures have been maintained for more than 170 months, and continuously evaluated for their ability to regenerate complete plants. Chromosome analysis was performed with fragments of calluses collected from the surface of the embryogenic cultures, and the squash preparations were stained by the Feulgen's and C-banding methods.

Results and Discussion

Embryogenic cultures were induced and have been maintained sucessfully since March 2003 (Figure 1). Calluses were analysed individually for numbers of aberrant and non-aberrant cells observed at initial, typical and final anaphases and telophases. The results demonstrate the occurrence of extensive mitotic abnormalities, mainly as a result of the formation of bridges involving chromosomes arms with heterochromatic knobs, that may lead to chromosome breakages (Figure 2). Mitotic abnormalities were mostly characterized by the occurrence of lagging chromosomes, chromosome bridges, chromatid fragments and micronuclei at telophases. The largest category of detectable aberrations involved breakage of knobbed chromosome arms. The initial event responsible for the occurrence of breakages and lagging chromosomes was characterized by the nondisjunction of newly replicated sister chromatids, which was observed to occur preferentially at the knob level.

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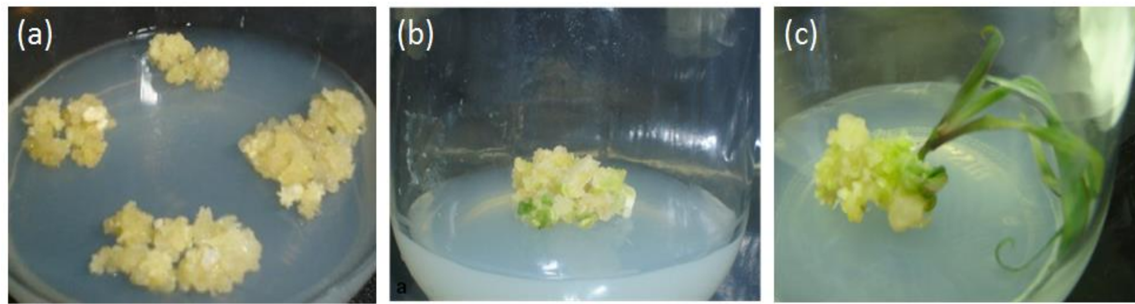

Figure 1: Cell cultures induced from immature embryos of the synthetic cultivar employed in this study, showing: (a) induction and maintenance of highly embryogenic cell cultures; (b) plant regeneration after transfering to a culture medium lacking auxin 2,4-D; (c) root formation in regenerated plants prior to transfer to soil.

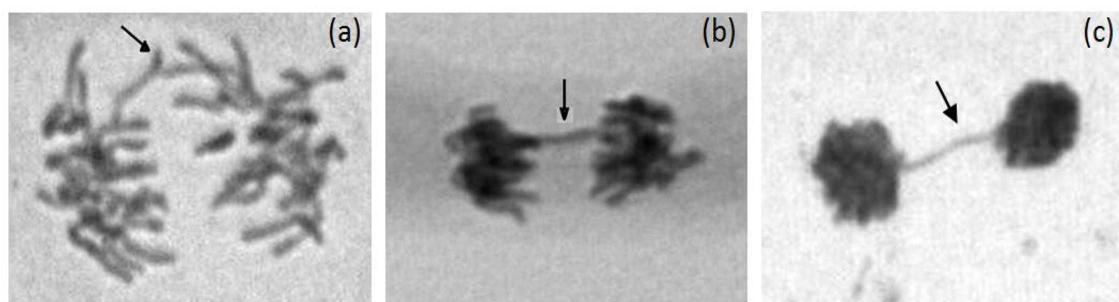

Figure 2: Photomicrographs of mitotic anaphases observed in cultured cells of the maize cultivar employed in this study (a) Typical anaphase showing the primary event, characterized by a delay in segregation of sister chromatids, at the heter ochromatic knob level; (b and c) Late anaphases showing single bridge, without heterochromatic knobs, apparently resulting from successive breakage-fusion-bridge cycles. Magnification: x100 objective; x10 ocular; x1.2 additional lens.

The analysis of C-banded anaphases showed that delayed chromatids were held together at heterochromatic knob sites (primary event), and the presence of typical bridges with and without bands corresponding to knobs. These events suggest the occurrence of breakage-fusion-bridge (BFB) cycles initiated by chromosome arms broken during the primary event, as effectivelly demonstrated by Fluminhan et al (1996a, 1996b) and Santos-Serejo and Aguiar-Perecin (2016).

It seems reasonable that the mechanism (or mechanisms) responsible for the generation of variations remains operative throughout the period of culture. This is reflected by the relatively constant number of mitotic figures showing evidence of the initial event – the delayed segregation of sister chromatids. The subsequent maintenance of aberrant cells over time could be due to rearrangements that create reciprocal translocations of the chromosome fragments that are produced from breakage events. Such mechanism could allow aberrant cells to survive, to proliferate and to compete with normal cells during the culture period. However, it should be emphasized that an effect of diplontic selection during cell proliferation might exist. The identification of the main causes of the mitotic abnormalities may indicate a possible way for their prevention.

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References

Aguiar-Perecin MLR de, Fluminhan A, Santos-Serejo JA, Gardingo JR. 2000. Heterochromatin of maize chromosomes: structure and genetic effects. Genetics and Molecular Biology 23 (4), 1015-1019.

Fluminhan A, Kameya T. 1996. Behaviour of chromosomes in anaphase cells in embryogenic callus cultures of maize (Zea mays L.). Theoretical and Applied Genetics 92 (8), 982-990.

Fluminhan A, Kameya T. 1997. Involvement of knob heterochromatin in mitotic abnormalities in germinating aged seeds of maize. Genome (Ottawa) 40 (1), 91-98.

Fluminhan Jr. A, Aguiar-Perecin MLR de, Santos JÁ dos. 1996a. Evidence for heterochromatin involvement in chromosome breakage in maize callus culture. Annals of Botany 78 (1), 73-81.

Fluminhan A, Ohmido N, Fukui K, Kameya T. 1996b. Heterochromatic knob-specific repeated sequence is associated with the formation of chromosome bridges in cultured cells and in germinating roots of aged seeds. Maize Genetics Cooperation Newsletter 70: 60-61.

Fluminhan A, Ohmido N, Fukui K, Mizugaki M, Kameya T. 1997. Visualization of methylated cytosine in maize somatic chromosomes. Maize Genetics Cooperation Newsletter 71: 75-76.

Green CE, Phillips RL. 1975. Plant regeneration from tissue cultures of maize. Crop Science, 15 (3): 417-421.

Kaeppler SM, Phillips RL. 1992. Tissue culture-induced DNA methylation variation in maize. Proc Natl Sci USA 90: 8773-8776.

Phillips RL, Kaeppler SM, Peschke VM. 1990. Do we understand somaclonal variation? In: International Congress on Plant Tissue and Cell Culture 7., Amsterdam, 1990. Progress in plant cellular and molecular biology. Proceedungs, NIJKAMP, H.J.J. and others ed. Dordrecht Kluwer Academic. P. 131-141.

Santos-Serejo JA, Aguiar-Perecin MLR de. 2016. Breakage–fusion–bridge cycles and de novo telomere formation on broken chromosomes in maize callus cultures. Genome 59 (6), 367-378.

Scandolieri RF, Koyanagui AP, Takahashi FT, Fluminhan A. 2006. Changes in chromosomes in highly embryogenic cultured cells and in germinating stored seeds of maize. Maize Genetics Cooperation Newsletter 80, 24-25.

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