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Amplification of heterochromatic knob size in callus culture by unequal sister chromatid exchange

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Tissue culture and in vitro plant regeneration systems have provided alternative means for mass proliferation of several plant species. Several reports have given evidence that successful plant regeneration from maize embryo-derived callus cultures is genotype dependent (see Fluminhan and Aguiar-Perecin, 1998). In addition, tissue and cell culture systems have been useful for studies on the effect of stress on chromosome stability. Chromosome breakage associated with heterochromatin regions has been observed in plant species, as for instance the occurrence of breakpoints on chromosome arms containing heterochromatic knobs detected by meiotic studies of regenerated maize plants (Lee and Phillips, Genome 29:122-128, 1987). In a previous study, we found altered chromosomes in embryo-derived callus cultures from sister lines obtained from a Brazilian flint variety. These materials were homozygous for knobs at the long arm of chromosomes 6 (K6L2; K6L3) 7 (K7L) and 8 (K8L), and the short arm of chromosomes 7 (K7S) and 9 (K9S); in one of these lines, K9S was not present. Chromosome changes were detected by C-banding technique applied to callus cells. Chromosome 7 was the most affected, and this was interpreted as a consequence of the presence of knobs on both arms of this chromosome (Fluminhan et al., Ann. Bot. 78:73-81, 1996). The presence of an altered chromosome 7 with a normal long arm and a duplication on the short arm (displaying two knobs), was explained by the occurrence of a breakage event at K7S followed by cycles of breakage-fusion-bridge (BFB). Interestingly, this abnormal chromosome was stable for several months in vitro, giving evidence that healing at the chromosome broken ends had occurred. In fact, it was further demonstrated the presence of telomeric sequences on the termini of this chromosome (unpublished). Other type of change observed in the chromosome 7 was the occurrence of amplification of the knob located on the long arm.

The origin of this amplification was investigated in further experiments, by the cytogenetic analysis of R_1 progenies resulting from regenerated plants derived from a callus culture designed 12F, obtained from line 13342/5. Figure 1 shows C-banded mitotic prophases of regenerated plants, respectively homozygous for the normal K7L

(Figure 1A) and heterozygous for the presence of the amplified K7L (Figure 2B). Fourteen heterozygous plants were selfed and in the progeny, 19 amplified K7L homozygotes, 58 heterozygotes and 39 normal K7L homozygotes were recovered. The plants homozygous for the K7L amplification survived. So, we interpreted that the amplification of K7L would not have been derived from a BFB event as it was the case of the change mentioned above involving the terminal knob at the short arm of chromosome 7. The knob on the long arm is not terminal and a breakage followed by BFB cycles would cause deletion of a significant distal region of the arm. In a further experiment using 2-4 month-old callus cultures derived from sister lines designated 13342/1, 13342/5, 132331 and their hybrids (references on the lines in Fluminhan and Aguiar-Perecin, Ann. Bot. 82:569-576, 1998), we observed metaphase cells with one of the homologues of chromosome 7 displaying an asymmetric C-band corresponding to K7L (Figure 1C). This gave evidence that unequal sister chromatid exchange at the knob site occurred in culture, and would modify the knob size without disrupting gene linkage in the chromatids involved. The frequency of this event was very low: it was detected in three cells of two lines and one hybrid in an experiment in which 5223 C-banded metaphases were analyzed and 2.35% presented alterations (knob amplification or reduction) on the long arm of chromosome 7. These results are interesting not only in the context of effects of tissue culture on heterochromatin, but also as evidence of one of the mechanisms that must have occurred during the evolution of maize races. For example, in a classical analysis of maize races, McClintock, Kato and Blumenschein (Chromosome Constitution of Races of Maize, Chapingo, México, 1981) characterized several genotypes by their knob position and sizes. This size polymorphism might have originated from unequal crossing–over involving germ cells.

Callus culture techniques and C-band preparations were carried out as previous described (Fluminhan et al., Ann. Bot. 78:73-81, 1996).

Figure 1. C-banded mitotic prophases of plants regenerated from a two-year-old callus culture designated 12F (A, B) and C-banded mitotic metaphase of a 2-month-old callus culture derived from hybrid 13342/5 x 13342/1 (C). Note the normal size of K7L in both homologues in A and a K7L amplification in B. The small arrow in C points to chromosome 7 showing an asymmetric band on the long arm and the large arrow indicates normal chromosome 7. Scale bar = 10μm.