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BRIEF COMMUNICATION

Chromosomal Instabilities in Callus Tissue from Haploid Barley (*Hordeum vulgare* L.)

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Abstract. Callus culture was derived from haploid barley embryos after crossing with *Hordeum bulbosum*. The callus tissue is cytologically heterogeneous, containing haploid, diploid and polyploid cells. Aneuploidy and karyokinetic irregularities were also observed. Some problems of chromosomal instabilities in plant tissue cultures are discussed.

A haploid cell culture is an excellent system for studying somatic plant cell genetics. However chromosomal instabilities occur mainly in plant tissue cultures (Sunderland 1973).

The present paper deals with chromosomal characteristic of the callus culture derived from haploid barley embryos.

Haploid embryos were obtained through crossing *Hordeum vulgare* (cv. Chiro Chingo) with *H. bulbosum* (diploid strain GBC 77). The procedures of crossing and embryo culture have already been described (Novák *et al.* 1977). A spontaneous callus growth was observed in 20 embryos extirpated at the age of 12 days and cultured on the medium C-17 (Jensen 1977) without growth regulators. After 30 days the calluses were transferred onto the medium B-5 (Gamborg *et al.* 1968) enriched with 5 mg l^{-1} 2,4-D and 500 mg l^{-1} yeast extract (Novák and Adamusová 1974), where they went on growing continually. After 120 days of *in vitro* culture we carried out a chromosomal analysis of callus cells. The callus pieces were taken at the beginning of the exponential growth stage, *i.e.* 10 days after the transfer onto the fresh medium. The tissue pieces were pretreated with 0.002 M oxiquinoline for 4 h, fixed with 1 : 3 acetic ethanol, hydrolysed in 1 N HCl for 10 min at 60 °C, macerated in 10% Bistrin, containing pectinase for 20 min and stained with leuco-basic fuchsine. 450 metaphase plates were evaluated.

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The callus tissue consists of parenchymous and meristemic cells. The mitotic activity occurred mainly in both cell types. Giant cells, tracheary elements and non-viable necrotic cells formed 40—60 per cent of the cell population.

The frequency of dividing cells ranged between 0 to 7.3% in individual samples, the mean value being 0.8% mitotic cells. There were indications of a partial synchronization of the division between adjacent cells.

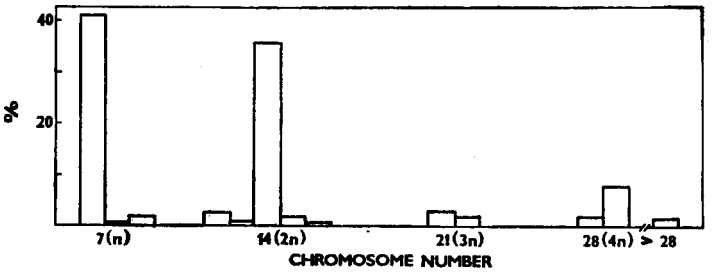

Fig. 1. Frequency of cells with different chromosomal numbers in callus tissue after 120 days *in vitro* cultivation. B-5 medium with 5 mg l^{-1} 2,4-D and 500 mg l^{-1} yeast extract.

Haploid chromosome number remained unchanged in 41% callus cells, while 35.5% cells were diploid and 8.8% tetraploid (Fig. 1.). In 14.7% cells we observed aneuploid (ranging from x—2x to 2x—4x), triploid and higher than tetraploid chromosome numbers. Frequency of different chromosomal classes in callus cells is shown in Fig. 1. Besides the changes in chromosome numbers we recorded structural rearrangements and chromosomal fragments. A detailed study on karyologic changes will be the objective of another paper. Multinucleate cells (about 3%) also occurred in the callus tissue. Micronuclei gave evidence of karyokinetic irregularities in the course of callus cell division.

Present results are in agreement with the observations of SCHEUNERT and his co-workers (SCHEUNERT *et al.* 1977, 1978, SHAMINA *et al.* 1978) on karyologic instability of barley callus tissue derived from diploid somatic cells. These authors recorded the differences in the frequency of polyploid cells in two callus strains differing from each other in growth and regeneration abilities. Our callus strain is characterized by a rapid growth and friability.

Endoreduplication and endomitosis play an important role in inducing polyploidy in many cells *in vitro* (*cf.* D'AMATO 1977). SCHEUNERT *et al.* (1978) stated that chromosomal status, growth and development of barley tissue culture was under the strong influence of the genotype of the initial cultivars which the callus strain had been derived from. This kind of dependence was found in other species, too (NOVÁK 1974).

Another factor, which can have a striking influence on chromosomal behaviour of callus tissue is ploidy degree of the initial explants. As shown in *Crepis capillaris* (SACRISTÁN 1971) and tobacco (NOVÁK and VYSKOT 1975), haploid-derived tissue cultures are markedly less cytologically stable than those of diploid origin. Rare plants regenerated from a haploid barley-derived callus contained karyologic changes — aneuploids and polyploids (SAAL-

BACH and KOBLITZ 1977). Our callus strains were subcultured on the media in which 2,4-D was omitted and substituted by various concentrations of IAA and BAP. Shoots were not formed in any case, roots just occasionally, which gives evidence of the loss of regeneration ability of this callus strain.

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