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Karyological Features of Barley Callus Tissues Cultured *in vitro*†

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Abstract. Long-term callus cultures of two barley cultivars have been investigated at the cellular level. One slowly growing, root forming callus of the barley cv. Bulgarische Nackte consists of cells the majority of which contain the diploid chromosome number. Contrarily, a fast growing callus of the cv. Elgina having no regeneration potency at all, is highly polyploid and includes more than two thirds of aneuploid cells. In this callus strain, a great number of multinucleate cells has been found. Some problems are discussed with respect to the relationships between growth rate, karyological stability and regeneration capacity in long-term callus cultures of cereals.

Experimental data with respect to the karyological and cytogenetical features of cereal cells cultured *in vitro* have relatively seldom been published. In callus strains derived from wheat (*Triticum aestivum*, 2n = 42) all but one showed the diploid chromosome constitution, while cultured cells from aneuploid-mixoploid lines showed much more variable chromosome numbers (24 to 126; ASAMI *et al.* 1975). Investigations in sugar-cane (HEINZ *et al.* 1969) revealed that some strains preserved their "diploid" ploidy level, while other strains, though grown under the same cultural conditions, did not. NORSTOG *et al.* (1969) reported that endosperm tissue cultures of ryegrass remained nearly triploid over a time span of ten years, but chromosome anomalities have often been observed in these cultures. Studies of cultured tissues from different species of many families indicated that cell populations cultured *in vitro* are usually karyologically heterogeneous (SHAMINA 1975).

Based upon these findings, karyological examinations of the *in vitro* cultivated barley tissues described in part I (SCHEUNERT *et al.* 1977) and part II (SHAMINA *et al.* 1978) of this series seem to be useful. Our aim was to look for correlations between karyological characteristics of the cells and the physiological behaviour of the tissue (cytodifferentiation, plant regeneration *etc.*).

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Material and Methods

Details have been described in part I (SCHEUNERT *et al.* 1977) and part II (SHAMINA *et al.* 1978) of this series. It should be stressed that there was no colchicine treatment prior to fixation and microscopical inspection of the slides.

Results and Discussion

1. Slow Growing Tissue Strain of the Barley cv. Bulgarische Nackte (*Hordeum vulgare* L. s. l., convar. *hexastichon* ALEF. s. l.)

Mitosis was found to be regular and the majority of cells (approx. 80%, Fig. 1) showed the diploid chromosome number (Fig. 2a). When polyploid cells were found (Fig. 2b), only a low number of changes of whole genomes was

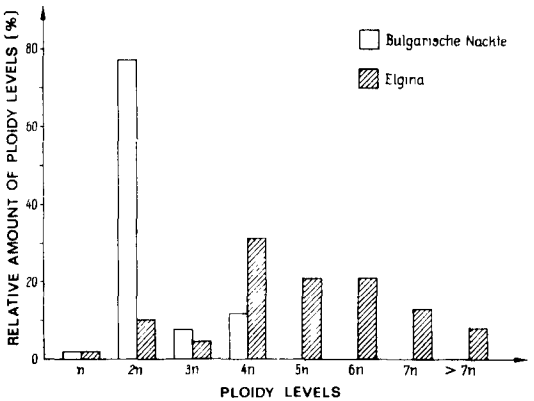

Fig. 1. The relative ploidy levels in the tissue strains of the cultivars Bulgarische Nackte and Elgina. Aneuploid cells (only scored in the case of 'Elgina' were counted in the next higher ploidy level.

The figure is based on 252 clearly countable metaphase plates for the inspected barley strain 'Elgina' and 102 for 'Bulgarische Nackte', respectively.

observed. The degree of compaction of chromosomes during mitosis was the same or only slightly larger than in root tip meristems (SMITH 1951). Multinucleate cells have very seldom been found and if so, then only in cells of the parenchymatic type (*cf.* SHAMINA *et al.* 1978).

2. Fast Growing Tissue Strain of the Barley cv. Elgina (*Hordeum vulgare* L. s. l., convar. *distichon* ALEF. s. l.)

Contrary to the situation found in the slow growing tissue strain of the cv. Bulgarische Nackte all tissue cultures derived from this strain were mixoploid, preponderantly tetraploid to more than heptaploid (Fig. 1, 3b—d), two thirds of all cells have been found to be aneuploid (Fig. 3e, f). These aneuploid cells may undergo regular mitosis (Fig. 4) but also *C*-mitosis (about 30%) has been observed. Metaphase chromosomes of such *C*-mitotic cells were supercontracted and their lengths were reduced to 1/2 or 2/3 as compared with root tip chromosomes (SMITH 1951). Furthermore, this is no normal arrangement of the chromosomes on the metaphase plate. The ratio of cells being in the pro-, meta- and anaphase (including telophase), re-

spectively, amounts in the normal (root tip) to about 3 : 1 : 2 (KÜNZEL 1968, BENNETT and FINCH 1972), their ratio is shifted to approximately 1 : 1 : 1. This is indicative of a prolonged metaphase which is characteristic of *C*-mitosis and supports the view that polyploid cells originated from restitution nuclei. From these results and from the occurrence of non-disjunctions and lagging chromosomes (Fig. 5) it may be presumed that the spindle apparatus of these cells is inactive or functionally impaired.

Another characteristic feature of this tissue strain is the presence of multinucleate cells (about 30%, Fig. 6). These findings may be explained by impairment of cytokinesis which has been found in various callus tissues (GUPTA and GADGIL 1973, MAHLBERG *et al.* 1975, KALLAK and YARVEKYLG 1977). But there are also other possible ways, *e.g.*, cells with more than one nucleus can originate by the formation of a multipolar spindle (Fig. 7, BÖÖK 1945). In this case the formation of cells with seven chromosomes should also be possible (Fig. 3a), especially if one takes into consideration that cells with 7 chromosomes need not necessarily represent cells with a complete haploid chromosome set. On the other hand, agglutinated nuclei in multinucleate cells (Fig. 6) may originate by merostathmokinesis (RIEGER 1963) in as much as after a slight and incomplete separation of the chromatids the formation of the restitution nucleus may follow.

Important problems connected with the causes of karyological irregularities in long-term tissue cultures are still unsolved. In the past, many reports described various karyological deviations occurring in cells cultured *in vitro* (*i.e.* KAO *et al.* 1970), but none of these has been satisfactorily explained as to its causes. Frequently, the opinion has been expressed that there may exist some trigger substances which are able to produce such changes of karyological features in long-term cultures, *e.g.*, polyploidization. In some experiments, we tried to look for substances possibly acting in this direction. As a test material the cytologically stable tissue strain of 'Bulgarische Nackte' was used.

Coconut water (REINERT and TORREY 1961) (tested in a range of 5% to 20%, with or without addition of growth promoting substances) did not cause polyploidization in this tissue strain. The same was true of kinetin (D'AMATO 1964, TORREY 1961) (tested in a range of 0.001 to 8 ppm) and benzylaminopurine (in the same concentrations). An exogenous addition of these cytokinins to the nutrient medium always resulted in necrosis of the barley cultures. Finally, 2,4-dichlorophenoxyacetic acid (MELCHERS and BERGMANN 1958, BAYLISS 1973) (used in stock cultures at 2 ppm and tested in a range of 0.5 to 40 ppm) has been applied as a growth stimulating component in all media used for barley callus cultures, both for the cytologically stable and unstable strains; no differential action on the cytological features of cells of the two strains has been observed (*cf.* also SINGH and HARVEY 1975).

The results obtained together with observations as to the regeneration capacity of barley callus cultures (part I; SCHEUNERT *et al.* 1977) and data of other investigators working with callus cultures of cereals indicate that fast growth is intimately connected both with karyological instability and absence of any regeneration capacity, and *vice versa*.

These correlations largely seem to be uninfluenceable physiologically, but

are strongly dependent on the genotypes and the cultivars used. On the other hand, an uncoupling of these correlations is expected to be extremely useful with respect to the future progress of work with *in vitro* cultured cells of cereals.

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Figures at the end of the issue.

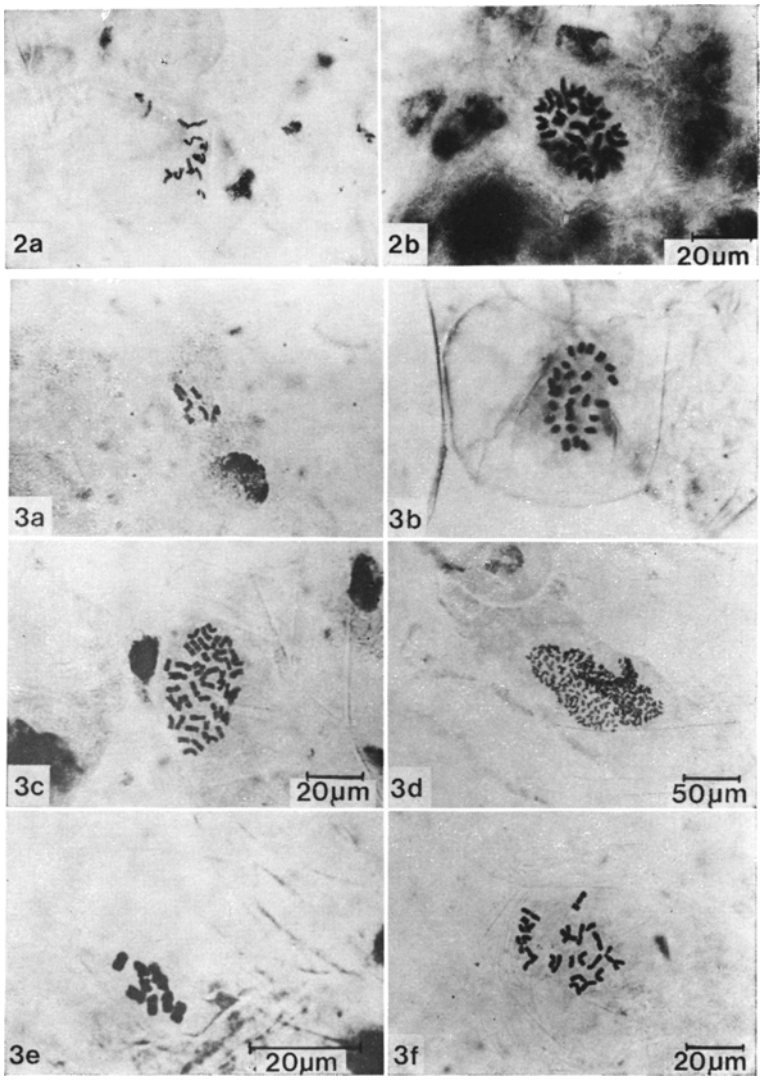

Fig. 2. Cells of different ploidy levels from 'Bulgarische Nackte'. a = diploid cell; b = tetraploid cell.

Fig. 3. Cells of different ploidy levels from 'Elgina'. a = "haploid" cell; b = tetraploid cell; c = heptaploid cell; d = highly polyploid cell; e, f = aneuploid cells.

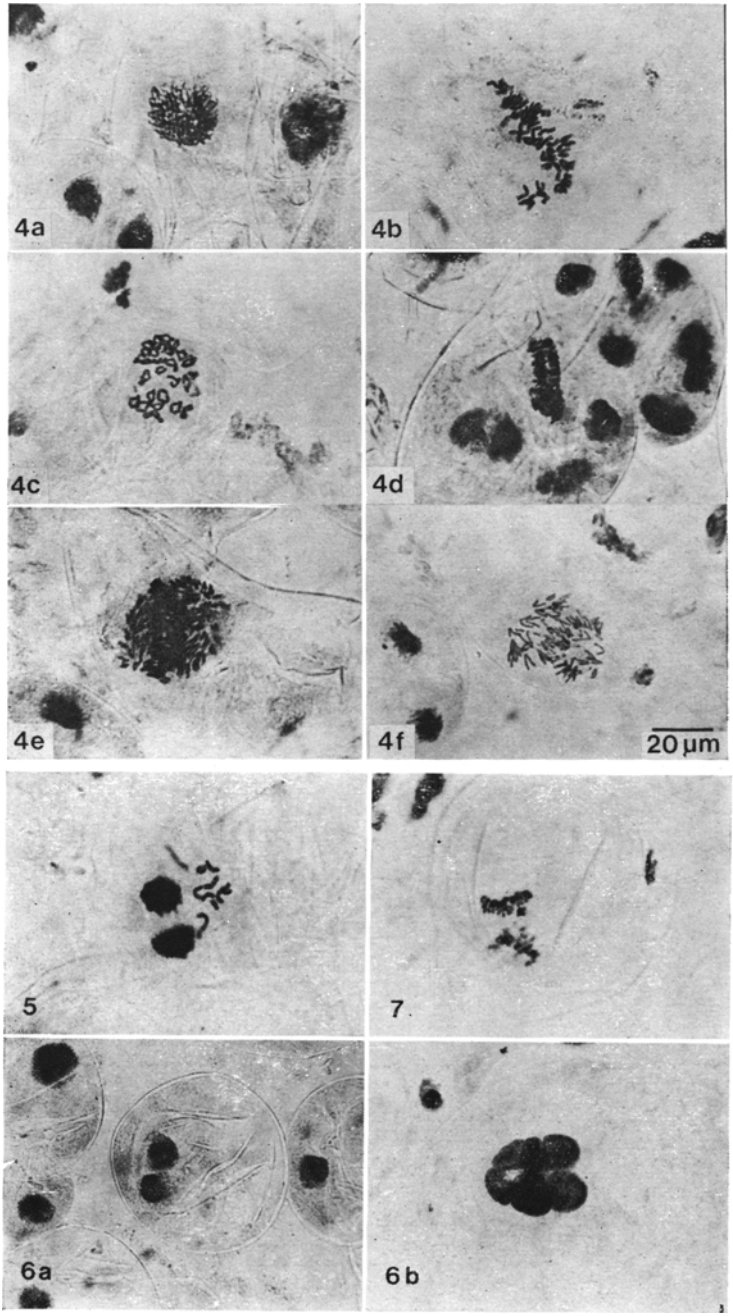

Fig. 4. Mitotic figures with fully functional spindle apparatus. a = prophase; b = metaphase; c, d = initiate anaphase; e, f = anaphase.

Fig. 5. Lagging of chromosomes in the case of the cv. Elgina.

Fig. 6. Multinucleate cells from 'Elgina'. a = binuclear cell; b = "agglutinate" multinuclear cell.

Fig. 7. Tripolar spindles observed in 'Elgina'.