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BRIEF COMMUNICATION

Anther Cultures of Maize

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Abstract. The presence of synthetic auxins (2,4-D at a concentration as low as of 0.5 to 5.0 mg l^{-1}, NAA at least at 5 mg l^{-1}) in the cultivation medium was essential for the induction of callogenesis in anther cultures of *Zea mays* L. The application of IAA was ineffective. Kinetin induced bursting, darkening and a rapid anther necrosis, but at an appropriate concentration ratio with 2,4-D it stimulated pollen maturation at the same time.

The regeneration of plants from microspores in culture represents the most effective method so far for obtaining haploid individuals as an initial material for breeding. However, the reliability of this technique, limited only to certain plant species, especially of the *Solanaceae* family, is a serious obstacle not yet elucidated, which prevents a general application of this method. The results of experiments with many plants of economic importance, especially cereals, are mostly negative or irreproducible (see a survey by CLAPHAM 1977), not excepting maize (the only paper by MURAKAMI *et al.* 1972). The aim of our work is a complex study of the conditions for callogenesis or embryogenesis induction in anther cultures of cereal plants, and those of the factors, both physiological and genetic, controlling morphogenic processes in this material. The results of our initial experiments with maize anther cultures, dealing with the determination of the conditions for the aseptical anther sampling and fundamental characteristics of their response to media of various hormonal composition, are the subject of the present communication.

Flowering plants of *Zea mays* L., hybrid CE 250 (Research Institutes of Plant Production, Praha), served as initial material. Anthers were sampled at the stage of mononucleate microspores. Owing to the irregular maturation of panicle spikelets, the presence of earlier (seldom later) developmental stages cannot be excluded, but from the point of view of the comparability

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TABLE 1

The effect of hormonal composition of media on the response of maize anthers in culture (Evaluated after a 12-week cultivation)

Medium number	IAA	NAA	2,4-D	Kinetin
1	—	—	—	—
2	5	—	—	—
3	2.5	2.5	—	—
4	—	5	—	—
5	—	2.5	2.5	—
6	—	—	0.5	—
7	—	—	5	—
8	—	—	0.5	2
9	—	—	5	1
10	—	—	5	0.1
11	5	—	—	1

The composition of the basal MS medium and organic components — see the text. The concentration of growth substances given in mg l^{-1}.

of the effect of various media this variability is negligible. The cytological analysis of the anthers cultivated for several weeks revealed that mature pollen grains occurred only in media containing kinetin (variants 8—11, see Table 1). One may thus presume that microspore maturation took place only in cultures.

A preliminary surface sterilization of panicles (using ethylalcohol or Chloramin) proved unnecessary; manifold washing in sterile distilled water was sufficient to ensure an almost 100 per cent sterility of primary cultures.

Excised anthers (without filaments) were inoculated in media of a common basal composition: mineral components according to MURASHIGE and SKOOG (1962), organic components (in conc. l^{-1}): sucrose 30 g, inositol 100 mg, vitamins according to WHITE (1943), Difco Bacto Casamino Acids 1 g, agar 7 g, but differing in the supplement of growth substances (see Table 1). Anthers from both flowers of one spikelet, *i.e.* 6, or, in the case of a casual cytological checking of the degree of microspore maturation, 5 anthers, were inoculated simultaneously into one test tube containing 10 ml of the medium. In five repetitions of the experiment more than 5800 anthers were cultured. The cultures were incubated in darkness at 26 ± 1 °C, for at least 16 weeks.

In the first 3—4 weeks of cultivation the morphological changes could be observed only with anthers cultured in media with a higher kinetin concentration (var. 8, 9, 11). The characteristic features were a twisting and later a bursting of pollen sacs, darkening and subsequent necrosis of tissues (see Fig. 1). In approximately 20—40 per cent of anthers from var. 8 and 9 pollen grains fell off; they were mostly abortive, but also mature. The cultures grown in media without growth substances or with IAA only, did not exhibit any morphological alteration (growth, change in colour or shape) throughout the whole cultivation period. No pollen maturation was detected. Most anthers cultivated in media with 2,4-D, NAA or their combination, gave a similar response. However, within a period of 6—8 weeks, in approximately 10 per cent of anthers from these variants parenchymatous tissue proliferated from

Twisting Bursting	Callogenesis	Darkening Necrosis	Pollen maturation
—	—	—	—
—	—	—	—
—	—	—	—
—	+	—	—
—	+	—	—
—	+	—	—
—	+	—	—
+	—	+	+
+	—	+	+
—	—	—	—
+	—	+	+

the lines of dehiscence of pollen sacs and small clusters (of a few mm in diameter) of a soft callus were formed (see Fig. 2). Its origin (from microspores or somatic cells) is questionable and attempts at its subcultivation failed.

Applying various exogenous factors to normally developing microspores in anther cultures may result in an enhanced frequency of callogenesis or embryogenesis. However, in the case of our material investigated the treatment of cultures with low temperature (NITSCH and NORREEL 1972), or ethrel application (BENNET and HUGHES 1972) to plants several days prior to anther sampling, were not successful. Following the low temperature treatment no changes occurred in anther morphology, and Ethrel induced a complete abortion of microspores and a hardening of pollen sac tissues; however, these results are to be considered as preliminary.

The presence of synthetic auxins, especially 2,4-D, is often a limiting factor of the induction of callogenesis in tissue cultures of cereals (see a review by YAMADA 1977), should they originate from the stem, leaf or root. Maize is no exception in this respect (see *e.g.* SHERIDAN 1975 and our unpublished results). The growth inhibitory effect of cytokinins seems to be also material specific (OPATRNÝ, unpublished results); from the point of view of callogenesis induction the anther cultures gave similar response as the cultures of other somatic tissues of the same material. A more detailed verification of this material or genotype specificity (using a wider spectrum of line and hybrid material) and the determination of the gametic or somatic origin of the calli induced will be the subject of further investigation.

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Figures at the end of the issue.

BOOK REVIEW

BUTCHER, D. N., INGRAM, D. S.: **Plant Tissue Culture.** — The Institute of Biology's Studies in Biology no. 65, Edward Arnold (Publishers) Ltd., London 1976. Pp. 68, 1.50 Lstg.

A thin booklet by two Cambridge authors represents the 65th volume of the "Studies in Biology", which is published for the use of "teachers and students at school, college and university" by the Institute of Biology, Queens Gate, London. The edition itself covers the entire field of biology and is written in a popular form on a good professional and up-to-date level. The authors had a rather difficult task: to present basic knowledge covering the whole field of plant tissue culture in a text about ten times shorter than the usual length of contemporaneous monographs, moreover at the period of an intense development of this branch (the manuscript was completed in 1974). Therefore they could hardly avoid certain errors or inaccuracies, *e.g.* one cannot give complete information on the effect of growth substances on the induction of morphogenesis in cultures in a few sentences, when the results of the research in this field are still far from being unambiguous. Moreover, the year 1974 was rich in important scientific happenings; the knowledge of the proceedings of the Canadian Symposium on haploidy or of those of the World Congress of IAPTC in Leicester would cause certain changes, especially in the chapters dealing with the most modern methods, *i.e.* androgonesis *in vitro* and protoplast cultivation.

The text is divided in a "classical" way, according to the type of culture (Organs and Embryos; Callus Cultures; The Growth of Single Cells; The Isolation and Culture of Plant Protoplasts). The chapters on root cultures (Butcher) and plant-pathogen cultures (Ingram) are especially well compiled and give evidence that the authors have a direct experimental experience in these problems. The concluding chapter is devoted to methods. Because of its arrangement (including also instructions for simple physiological-morphological experiments) revealing pedagogical erudition of the authors, it represents the main positive quality of the booklet. In the list of the literature recommended British sources are excessively preferred. Regardless of the fact that it was published 15 years ago, the classical Gautheret's monograph should not be lacking. The quality of illustrations is mediocre; good photographs would well substitute for some of too schematic, space-wasting pictures (*e.g.* cell types from the carrot culture).

A conclusion may be drawn that the booklet gives a good example to specialists how to inform briefly and in a popular form of a wide problem; for laics it is an introductory literature of a good quality. If further publications of the series mentioned will be of this professional and formal level (some of the titles are indeed attractive for a botanist), British students and teachers are to be envied, indeed.

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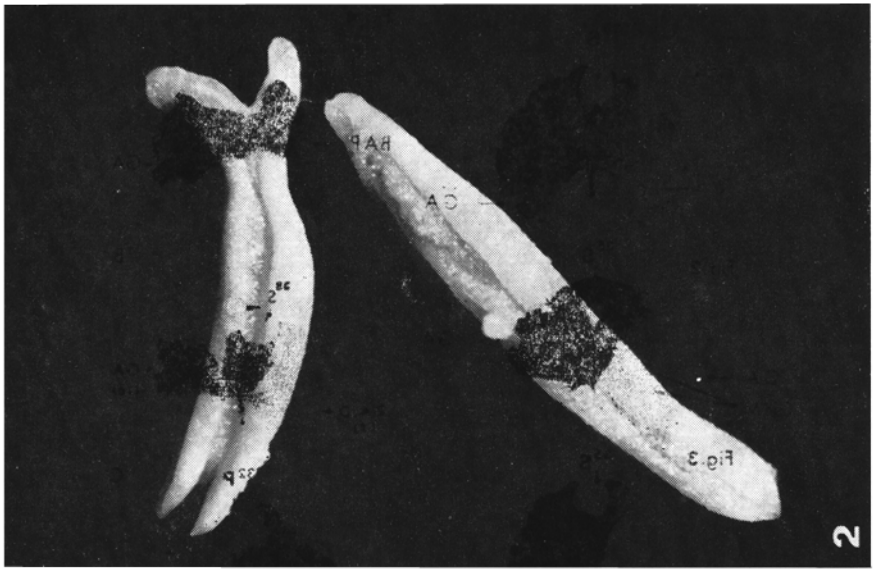

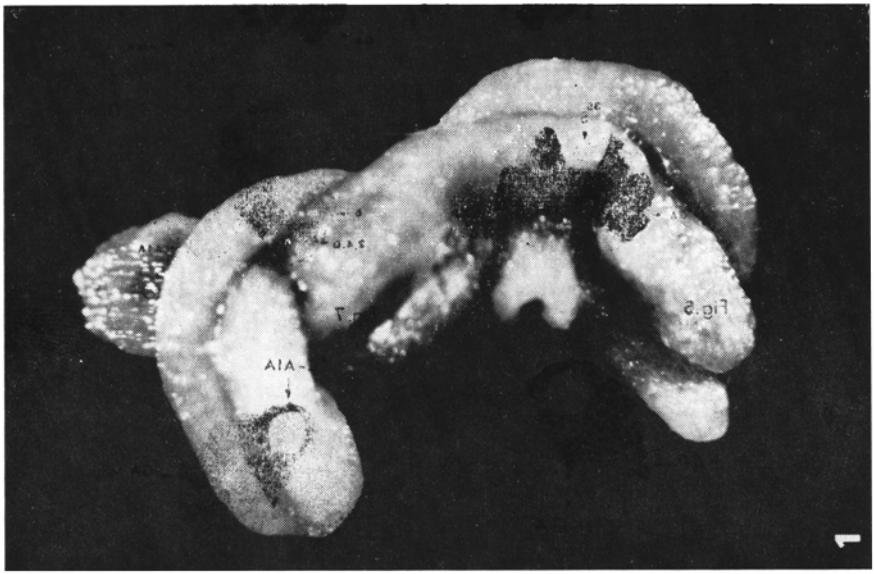

Fig. 1. The twisting and bursting of maize anthers after a 3—4 week cultivation in a medium containing IAA (5 mg l^{-1}) and kinetin (1 mg l^{-1}). × 25.
Fig. 2. The proliferation of callus tissue from maize anthers after an 8-week cultivation in a medium containing 2,4-D (5 mg l^{-1}). × 25.