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In vitro culture of *Cucumis sativus* L. VI. Histological analysis of leaf explants cultured on media with 2, 4-D or 2, 4, 5-T

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Abstract

The developmental sequence of callus initiation and somatic embryogenesis in leaf explants of *Cucumis sativus* cv. Borszczagowski was analysed and compared on media containing two different auxin phenoxy-derivatives (2, 4-D and 2, 4, 5-T) and cytokinin (BAP or 2iP). During the first 20 days of culture on media with 2, 4, 5-T proliferation of parenchymatic tissue occurred mainly and only small meristematic centers were observed. There was an intensive detachment of parenchymatic cells and dissociation of their cell walls near vessels and in the lower part of the explant adjacent to the medium. These cells were strongly plasmolysed. On the 2, 4-D containing medium mostly meristematic tissue developed, proliferating around vascular bundles and forming meristematic centers or promeristem-like structures. After 35-50 days of culture, secondary callus was formed by separation of meristematic cells from the meristem surface in explants cultured on the 2, 4-D containing medium. On medium supplemented with 2, 4, 5-T the detachment of parenchymatic and meristematic cells occurred, along with formation of a gel-like substance. The gel-like callus contained multi-cellular aggregates, proembryoids and embryoids. This type of callus tissue was initiated more intensively on medium with 2, 4, 5-T, but the frequency of somatic embryogenesis was much lower. The periferial cells of aggregates, proembryoids and embryoids showed the tendency to separate from the surface of the tissue. Many embryoids formed adventitious embryos.

Key words: *Cucumis sativus L., embryogenesis, leaf explants*

INTRODUCTION

Somatic embryogenesis in leaf explants of different *Cucumis sativus* cultivars occurs frequently on media containing cytokinins (BAP or 2iP)

Abbreviations: BAP — 6-benzylaminopurine, 2, 4-D — 2, 4-dichlorophenoxy-acetic acid, 2iP — γ, γ-dimethylallyl-amino-purine, 2, 4, 5-T — 2, 4, 5-trichlorophenoxy-acetic acid

and 2, 4-D (Malepszy et al. 1982) or 2, 4, 5-T (Malepszy and Nadolska-Orczyk 1983). A similar pattern of development has been induced on media with other auxin phenoxy-derivatives (such as 4-phenoxyacetic acid), whereas it was not observed during culture on media supplemented with IAA or NAA (unpublished data). After transfer of the whole explants or isolated embryoidal structures onto auxin-free or hormone-free medium, plantlets have been developed (Malepszy and Nadolska-Orczyk 1983, Nadolska-Orczyk and Malepszy 1985). Macroscopic observations showed that on media containing auxin phenoxy-derivatives, regardless of the type of cytokinin used, prior to plantlet development the initiation of primary callus and then secondary, gel-like callus occurred. The gel-like callus was initiated much earlier and most intensively during culture of explants on 2, 4, 5-T containing medium. On the other hand, the medium supplemented with 2, 4-D was much more effective in promoting plantlet development (unpublished data). On the latter medium the first embryoidal structures were formed about two weeks earlier than those derived from explants cultured on the 2, 4, 5-T containing medium.

In the present paper, by means of standard microscopic procedures, the histological changes taking place during culture of leaf explants of *Cucumis sativus* cv. Borszczagowski were analysed using media supplemented with the respective cytokinin and 2, 4-D or 2, 4, 5-T at concentrations which were found the most suitable for initiation of the gel-like callus and intensive development of plantlets.

MATERIAL AND METHODS

Leaf explants of *Cucumis sativus* cv. Borszczagowski were obtained according to the method described earlier (Malepszy and Nadolska-Orczyk 1983) and cultured on the Murashige and Skoog medium (1962) supplemented with 0.8 $mg \cdot dm^{-3}$ 2, 4-D and 0.8 $mg \cdot dm^{-3}$ 2iP or 1.2 $mg \cdot dm^{-3}$ 2, 4, 5-T, 0.8 $mg \cdot dm^{-3}$ BAP and 250 $mg \cdot dm^{-3}$ edamin.

Explants were maintained in the dark at a temperature of 26°C. After 10, 20, 35 and 50 days of culture the material was fixed and mounted in paraffin or epon resin (Luft 1961). Paraffin sections (10 μm thick) were stained in hematoxylin with ruthenium red, whereas for epon sections (4 μm thick) PAS staining with toluidine blue (Włoch 1981) was applied.

RESULTS

The main type of tissue initiated from leaf explants after 10 days of culture on the 2, 4-D and 2iP containing medium was a meristematic

PLATE I

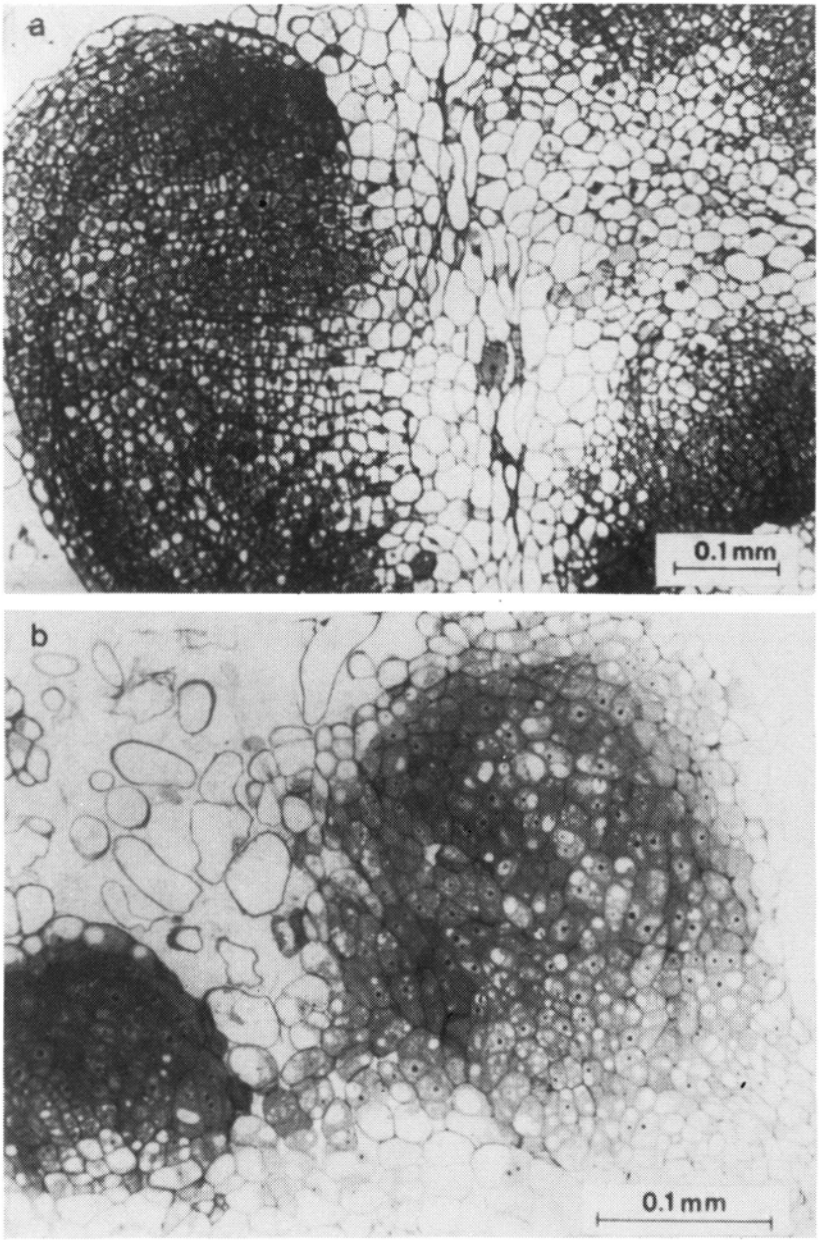

Fig. 1. Changes observed in leaf explants after 10 days of culture on media with 0.8 mg·dm^{-3} 2, 4-D and 0.8 mg·dm^{-3} 2iP. a — initiation of meristematic centers around vascular bundles; b — promeristem-like structures and large, detached, necrotic cells

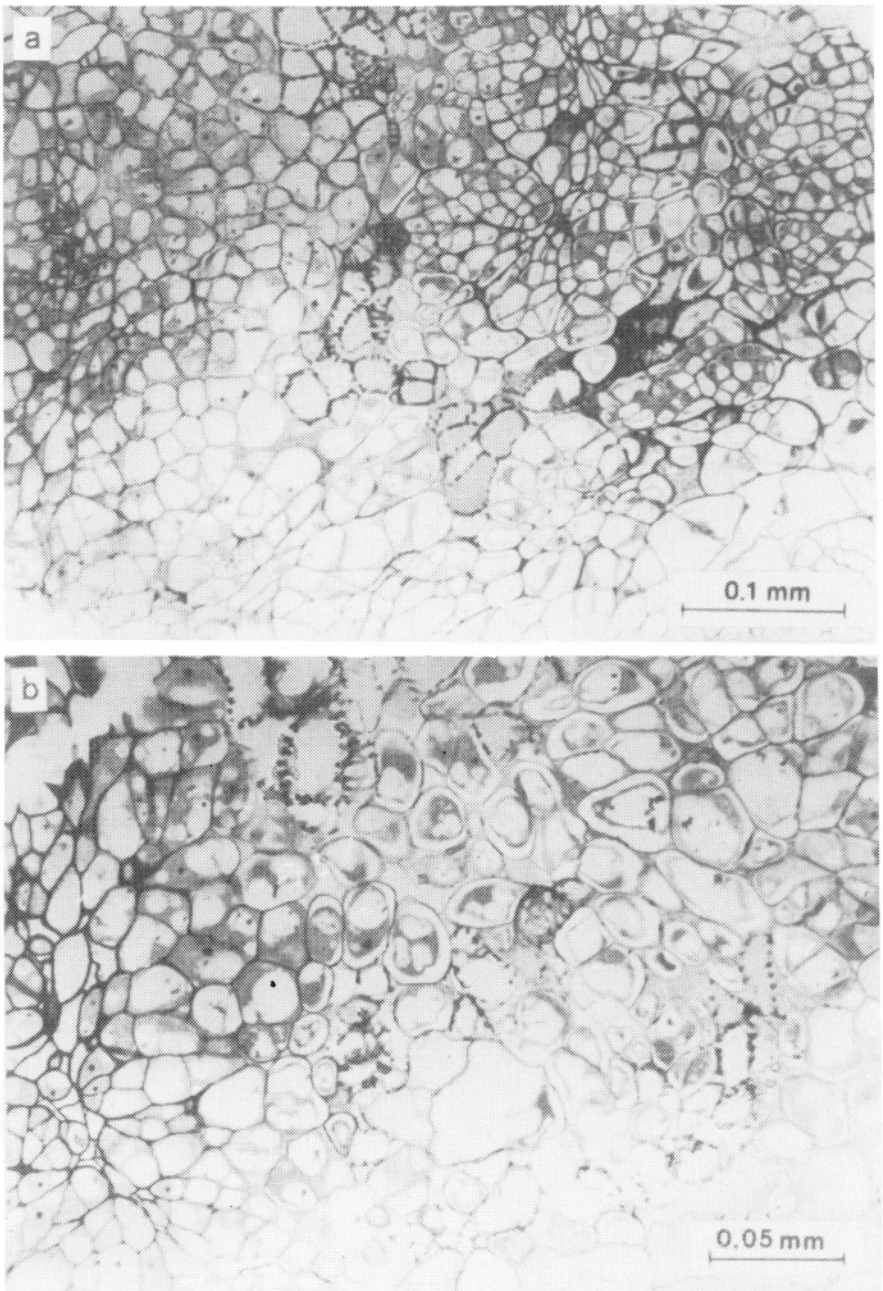

Fig. 2. Changes observed in leaf explants after 10 (a) and 20 (b) days of culture on media with 1.2 $mg \cdot dm^{-3}$ 2, 4, 5-T and 0.8 $mg \cdot dm^{-3}$ 6-BAP. a — proliferation of parenchymatic tissue in leaf explants; b — parenchymatic cells, strongly plasmolysed and desintegrating, visible in the vicinity of vessels

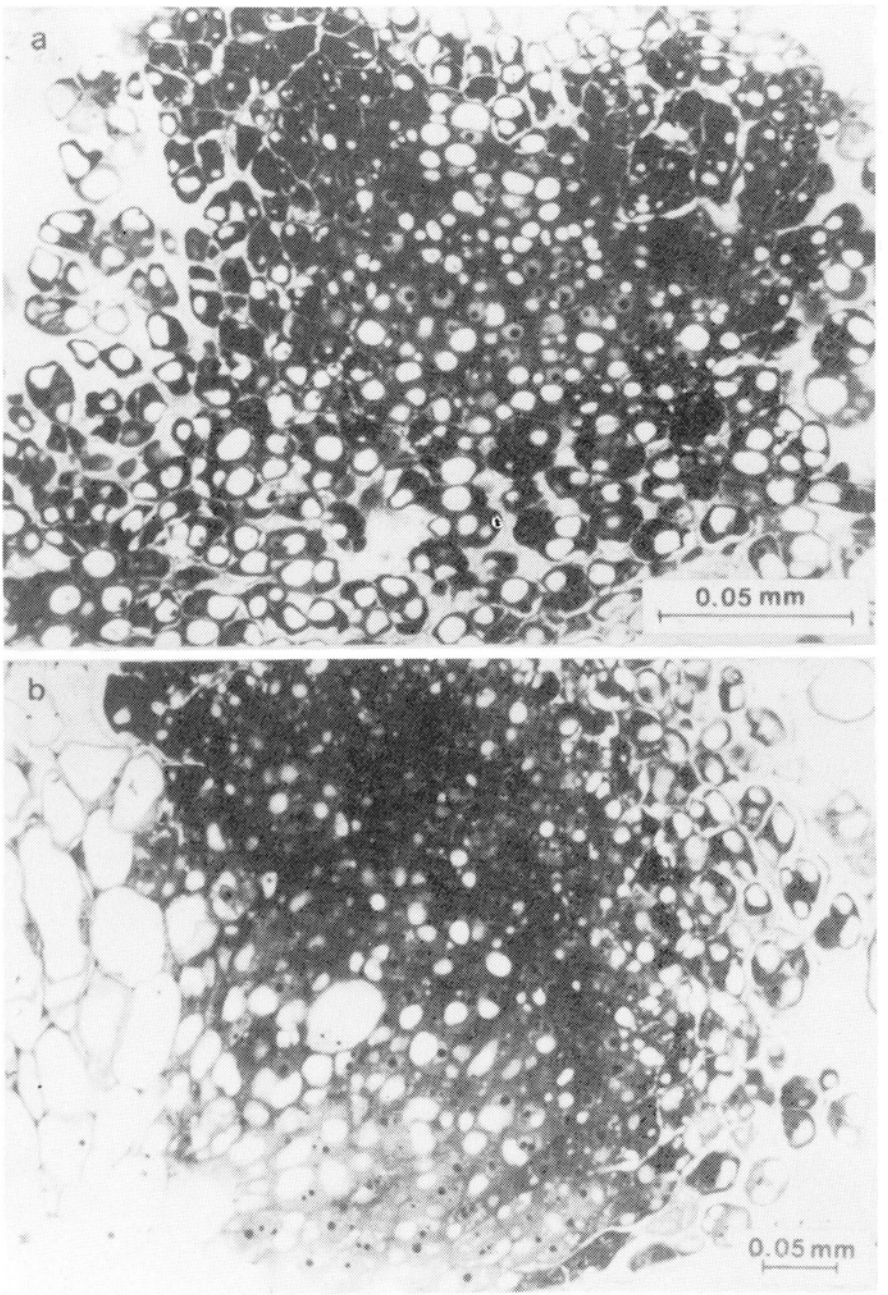

Fig. 3. 35 days of culture on media with 0.8 $mg \cdot dm^{-3}$ 2, 4-D and 0.8 $mg \cdot dm^{-3}$ 2iP (a) or 1.2 $mg \cdot dm^{-3}$ 2, 4, 5-T and 0.8 $mg \cdot dm^{-3}$ 6-BAP (b). Loosening and detachment of meristematic cells from the surface of meristems

PLATE IV

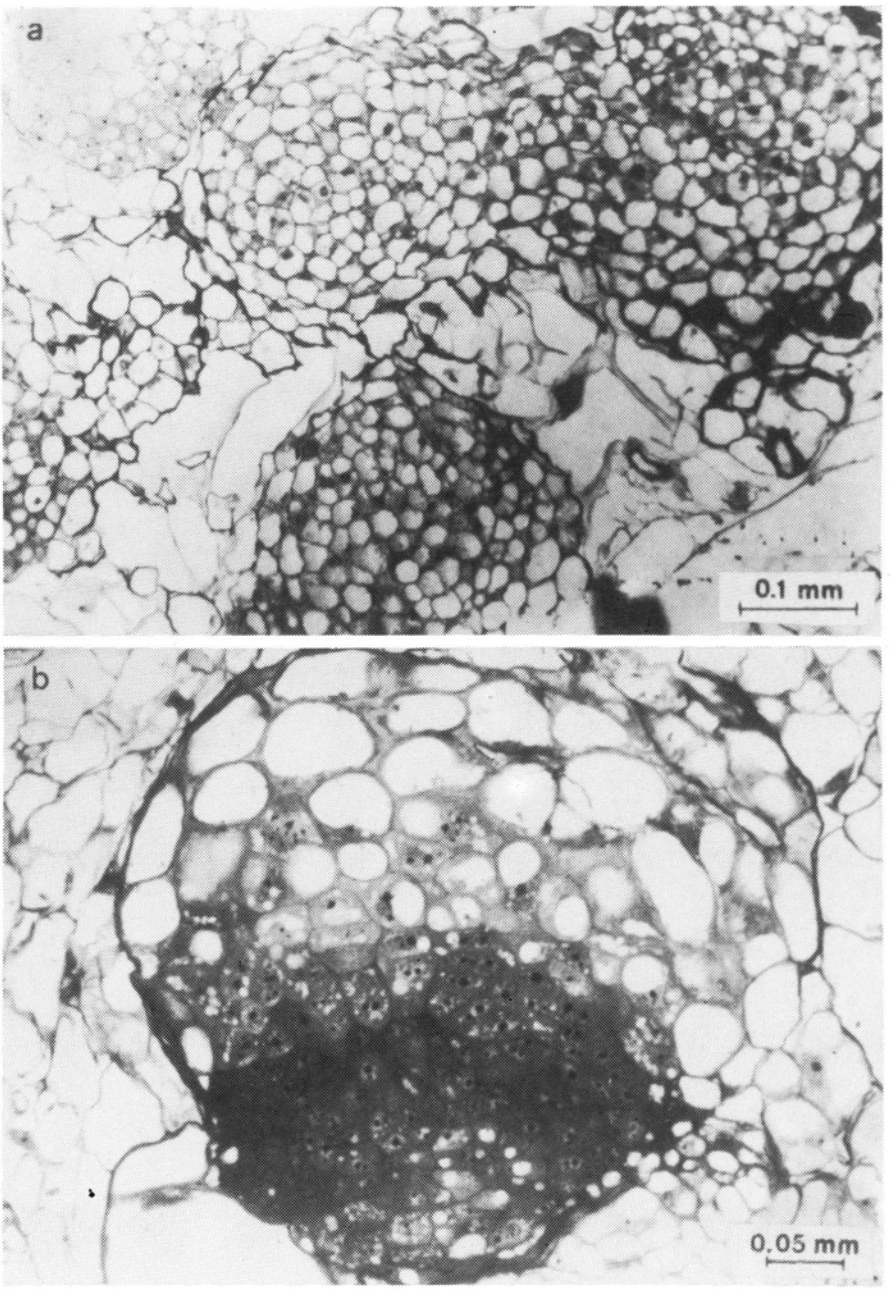

Fig. 4. 35 days of culture on media with 0.8 mg·dm^{-3} 2, 4-D and 0.8 mg·dm^{-3} 2iP (a) or 1.2 mg·dm^{-3} 2, 4, 5-T and 0.8 mg·dm^{-3} 6-BAP (b). a — gel-like substance containing globular embryoids; b — young embryoids

PLATE V

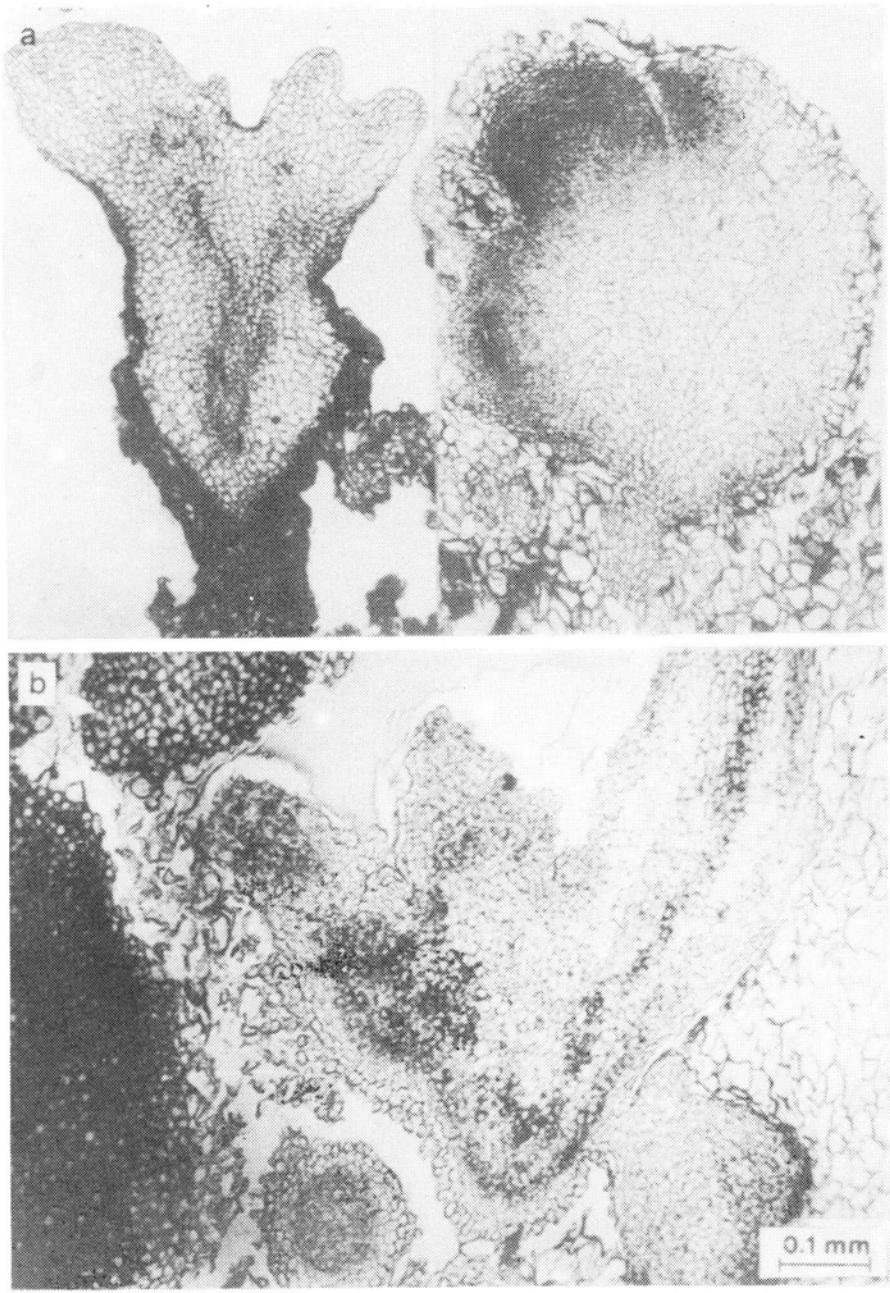

Fig. 5. 50 days of culture. a — various types of abnormal embryoids on media with 1.2 mg·dm^{-3} 2, 4, 5-T and 0.8 mg·dm^{-3} 6-BAP; b — mature embryoid with adventitious embryo on media with 0.8 mg·dm^{-3} 2iP and 0.8 mg·dm^{-3} 2, 4-T

type of callus which proliferated along both sides of the explants, forming meristematic centers (Fig. 1a), promeristem-like structures (Fig. 1b) or, occasionally smaller groups of meristematic cells. The parenchymatic tissue of the explants was partially disintegrated into single, large necrotic cells.

The explants cultured in the presence of 2, 4, 5-T and BAP (10th day of culture) showed mainly a proliferation of parenchymatic tissue. Single cells with deeply stained cytoplasm were observed in the subepidermal layer or, less frequently, near vascular bundles. The initial detachment of parenchymatic cells and formation of a gel-like substance was noted in the place the explants were excised and close to vessels (Fig. 2a).

After 20 days of culture on the medium with 2, 4-D and 2iP the extension of meristematic centers proceeded, whereas on that with 2, 4, 5-T and BAP the proliferation of parenchymatic cells and initiation of small meristematic centers continued. Moreover, intensive detachment of parenchymatic cells and dissolution of their cell walls along with formation of a gel-like substance was evident. The cells were strongly plasmolysed (Fig. 2b). These changes were the most pronounced at vessels and in the lower part of the explant, adjacent to the medium.

After 35 days of culture, the cross section surface of explants being maintained on 2, 4-D and 2iP containing medium revealed large and variably-shaped meristematic centers, separated by small groups of disintegrating parenchymatic cells. Around meristematic centers the loosing and detachment of cells, accompanied by the formation of a gel-like substance which stained red was noted (Fig. 3a). Simultaneously, in different parts of the explant, but most frequently on its peripheric layer and surface embryoids and proembryoids in various stages of development were present (Fig. 4a).

In explants maintained on 2, 4, 5-T and BAP containing medium (35th day of culture) mainly centers of parenchymatic tissue and only small groups of meristematic cells were present. In both cases, in the upper part of these centers, the process of loosening and detachment of cells, along with formation of the gel-like substance occurred (Fig. 3b). On this medium, the cells embeded in the gel were more variable than those culture in the presence of 2, 4-D and 2iP. The majority of them were irregular in shape and their cytoplasm was weakly stained. Also, the young stages of embryoids were much less frequent (Fig. 4b).

After 50 days of culture, the proliferation of meristematic cells (on the 2, 4-D and 2iP medium) and of parenchymatic and meristematic cells (on the 2, 4, 5-T and BAP medium) continued, as did their detachment. On both media embryoids in more advanced developmental stages were frequently seen. Many of them formed adventitious embryoids. Cells present in the peripheral part of the proembryoids were detached (Figs. 5a and 5b).

On the 2, 4, 5-T and BAP containing medium, the embryoids displayed more variable shapes and the process of cell detachment was more intensive.

DISCUSSION

According to Caldas (1971, cf. Sharp et al. 1980) the epidermal stem, hypocotyl and embryo cells can initiate secondary embryogenesis without transitory callus stage. On the other hand, as far as the cells of bark, xylem and floem are concerned, such a stage is required during differentiation to achieve a proper sequence of stages leading to embryo formation.

The results of our histological studies seem to show that in cucumber leaf explants cultured on media containing cytokinin and 2, 4-D or 2, 4, 5-T, the initiation of somatic embryogenesis proceeds by expansion of meristematic centers and formation of very friable, gel-like callus. Such a type of callus is formed the earliest and most intensively in explants cultured on media supplemented with 2, 4, 5-T. However, there seems to be no direct relationship between the initiation of the gel-like callus and the frequency of somatic embryogenesis. This can be explained by a slightly different influence of 2, 4, 5-T combined with cytokinin on the tissue of the explant, resulting in weaker predetermination of tissue to forming meristematic cells (as is observed in presence of 2, 4-D and cytokinin), and this combination of growth regulators induces more intensive divisions of parenchymatic cells.

One of the well known effects of 2, 4-D is tendency to break down cell clumps due to the disappearance of fibre in the middle lamella (Halperin and Jensen 1967). In our studies, a similar effect was observed, namely the cells were seperated from each other with a simultaneous formation of a gel-like substance. Moreover, in explants growing on media supplemented with 2, 4-D the cells in the gel were probably derived exclusively from meristematic tissue, whereas in those cultured in presence 2, 4, 5-T they were both of parenchymal and meristematic origin. The latter could be another cause of the weak tendency towards somatic embryogenesis on this medium.

In our material, the initiation of meristematic centers started probably from cells of bundle sheath around vessels. It is evidenced by the radial extension and close adherence of meristematic centers to the bundle cells as well as by the presence of parenchymatic tissue between vascular--meristematic centers remnants of the epidermal layer.

The repeating cycle of de- and redifferentiation on auxin containing media is well known phenomenon (Konar and Nataraja 1969, Zee

and Wu 1979). A similar pattern was also noted in our studies. The influence of cytokinin and 2, 4-D or 2, 4, 5-T on the division of meristematic centers, the detachment of cells from the surface of these centers and from embryoids, as well as the development of adventitious embryoids accurred as long as the culture was maintained on media supplemented with these growth regulators.

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Kultura Cucumis sativus L. in vitro. VI. Analiza histologiczna eksplantatów liściowych na pożywce zawierającej 2, 4-D lub 2, 4, 5-T

Streszczenie

Analizowano i porównywano sekwencję rozwoju kalusa i embryogenezy somatycznej w eksplantatach liściowych *Cucumis sativus* var. Borszczagowski na pożywce MS zawierającej dwie różne fenoksypochodne, tj. 2, 4-D lub 2, 4, 5-T oraz cytokininę (6-BAP lub 2iP). Przez pierwsze 20 dni kultury na obu pożywkach obserwowano różne wzory rozwoju:

1) Na pożywce z 2, 4, 5-T następowało głównie rozrastanie się tkanki parenchymatycznej oraz bardzo małych obszarów merystematycznych. W pobliżu naczyń oraz w dolnej części eksplantatu przylegającej do pożywki zachodziło silne oddzielenie komórek parenchymatycznych oraz rozpuszczanie ich ścian komórkowych. Komórki te były silnie spłazmolizowane. 2) Na pożywce z 2, 4-D obserwowano najczęściej wytwarzanie tkanek merystematycznych, które rozrastały się wokół wiązek przewodzących, tworzące obszary merystematyczne oraz struktury przypominające promerystemy.

Po 35–50 dniach tworzył się kalus wtórny, poprzez oddzielanie się komórek merystematycznych z powierzchni merystemów na pożywce z 2, 4-D, oraz komórek merystematycznych i parenchymatycznych na pożywce zawierającej 2, 4, 5-T z jednoczesnym wytwarzaniem substancji śluzowatej. W wytworzonym żelopodobnym kalusie spotyka się wielokomórkowe agregaty, proembrioidy i embrioidy. Kalus żelopodobny jest wytwarzany znacznie intensywniej na pożywce zawierającej 2, 4, 5-T ale znacznie słabiej zachodzi na niej proces embriogenezy somatycznej. Komórki znajdujące się na zewnątrz agregatów, proembrioidów i embrioidów, oddzielały się. Wiele embrioidów wytwarzało embrioidy przybyszowe.