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OBSERVATIONS ON THE PROLIFERATION OF STEM PITH PARENCHYMA IN VITRO

I. General Introduction

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INTRODUCTION

The work of Gautheret (1935, 1939) and some of his collaborator, (e.g. Morel, 1948) has shown that cambium and its derivativels for instance xylem and phloem parenchyma elements, are suitabe for cultivation in vitro.

On the other hand White(1939 *a* and *b*) has proved that tumour tissue taken from a *Nicotiana*-hybrid is also able to thrive in vitro.

The work of these authors clearly shows that the internal structure of the cultivated products is, as a rule, a complex one, a not very surprising fact since cambium is destined to produce different types of cells. Furthermore, from the beginning both cambium and White's tobacco tumour tissue exhibited a certain complexity. The tissue cultures derived from such material show a disorderly arrangement of phloem and xylem elements embedded in parenchyma that is traversed by meristematic zones. In only a few cases a tissue culture consisting solely of parenchymatous cells has been derived from cambium (Gautheret, oral communication, Morel, 1948). In our own laboratory such a tissue culture has been obtained from a cambium proliferation of *Rosa banksiopsis* Bak.

It is perhaps advisable to discuss the term "differentiation". Weiss (1949) defines differentiation as the "unidirectional changes in the character of cells and cell generations during their life history, transformations by which they become increasingly different from their former selves, their parent cells, and the cells of other strains which have taken divergent courses". In this way differentiation is "unidirectional, progressive, and irreversible".

It is certainly not our intention to use the term differentiation in this sense. As a matter of fact, it is still quite questionable whether any plant cells exist which show differentiation in the way Weiss defines this for vertebrate cells, the more so where this author connects the occurrence of differentiation with the impossibility of dedifferentia-

tion, "the actual return to a state of wider potency, i.e. the recuperation of something that had ostensibly been lost".

For plant cells BUVAT (1944, 1945, 1951) describes a physiological differentiation followed by a morphological one, i.e. the formation of metabolic products, loss of activity to proliferate and reduction leading, finally, to extinction of histogenetical potentialities. According to Buvat dedifferenciation is possible. It is "le processus inverse de la différenciation, c'est un rajeunissement des cellules, qui leur fait recouvrir cequ'elles avaient perdu en se différenciant".

In order to solve the problem which of the definitions of the term "differentiation" applies it might be useful to have the disposal of strains of simple structure.

As mentioned before most plant tissue cultures exhibit a more or less complicated structure, even the parenchymatous strain of *Vitis vinifera* L. described by MOREL (1948) contains many tannin cells amidst tannin-free ones.

The first thing to do was to try to find some plant tissue suitable as a starting material. It was considered possible that a simple tissue might proliferate to give a strain with a similar structure. Pith parenchyma was chosen, first of all because the medulla of many plant stems has a simple structure. Secondly, medullar parenchyma might breed true, while this tissue "shows up" nearest to the apex, and therefore, might have lost a number of potencies.

On the other hand several plants show certain idioblasts in the pith dispersed between the "normal" cells. Proliferation of this type of medulla might give some information about differentiation in the sense Weiss implies.

SCOTT (1941) studied the ontogeny of the stem of *Ricinus communis* L. In this plant several types of idioblasts occur, e.g. cells containing druses of calcium oxalate and so-called fat idioblasts. These idioblasts are arranged in longer or shorter vertical rows, indicating that the cells of each row were sooner or later derived from one idioblast mother cell. BLOCH (1947) points out that the cells in question seem to have differentiated into a fixed state as they prove to breed true in wound tissue.

There remains the possibility that medullar parenchyma cells, as has been described for other cell types, have lost none of their potencies during the development of the stem and have retained the ability to dedifferentiate to a more or less meristematic state from which a variety of cells or tissues or even organs such as shoots or roots may arise (cf. BLOCH, 1941, 1944; BUVAT, 1944, 1945, 1951; HARTSEMA, 1926; NAYLOR and JOHNSON, 1937; PRIESTLEY and SWINGLE, 1929; SINNOTT and BLOCH, 1940). SIMON (1908) describes proliferation of pith followed by differentiation into parenchymatous and lignified elements in cuttings of *Populus*. ULEHLA (1928) mentions the appearance of new cell walls in discs of pith parenchyma of the giant cactus *Carnegiea gigantea* Britton and Rose which had been kept for three weeks on sucrose solutions. WHITE (1939*a*), however, did not obtain any growth in excised fragments of medullary parenchyma of tobacco.

KANDLER (1950) mentions the proliferation of pith parenchyma of stem segments of *Impatiens*; details about the internal structure of these outgrowths are not given. It is necessary to lay stress on the fact that in the experiments of Simon and Kandler the pith cells remained in organic contact with the cells of the surrounding tissues. GAUTHERET (1951) mentions the proliferation of medullary parenchyma of the rhizome of the Jerusalem Artichoke resulting in the formation of callus containing vascular elements. It is not clear, however, whether isolated parenchyma tissue without attached vascular tissues was used by the author. JABLONSKI and SKOOG (1954), finally, using high concentrations of indole acetic acid (2–3 mg/l) failed to obtain cell division in excised tobacco pith. Cell division occurred, however, in pith tissue with attached vascular strands, in severed pith tissue placed in contact with vascular tissues, or even in isolated pith parenchyma if water extracts of vascular tissues are added to the nutritive medium. The same is the case with coconut milk or malt extract. In the presence of these substances callus formation was obtained. The tissue thus obtained can be maintained indefinitely in subcultures, and is capable of differentiation to produce vascular strands.

At the moment we started our investigation the publications of Gautheret and of Skoog and his collaborator had not yet appeared. However, the results obtained seem to us sufficiently important to justify their publication.

MATERIAL AND METHODS

A number of plants were studied with regards to the presence of a well-developed and living pith body. Only some of these plants, however, proved to possess pith that, under the studied circumstances, could be successfully cultivated in vitro.

Very appropriate for the purpose were medullary parenchyma from the stem of the Jerusalem Artichoke (*Helianthus tuberosus* L.), in an unknown variety and in the variety White Jerusalem Artichoke, and from suckers of the Elder (*Sambucus nigra* L.) and its weeping variety *pendula* Dipp. Furthermore, the stem of a garden variety of *Zinnia elegans* Jacq., the long shoots of *Ginkgo biloba* L. and twigs of *Taxus baccata* L. var. *aurea* Carr. furnished pith that, proliferating in vitro, produced results worth mentioning.

To isolate sterile medullary parenchyma, the stems and twigs are defoliated and debudded, then firmly rubbed with a wad of absorbent cotton wool soaked in alcohol 96 %, and for about one hour and a half, put into glass cylinders filled with a solution of chlorine and stoppered with absorbent cotton wool. The chlorine solution was prepared in the usual way by intermittently stirring during ten minutes of 100 gms of commercial bleaching powder with 1000 cc of tap water followed by filtration.

In the case of *Ginkgo* and *Taxus* the twigs show suberization. After desinfection with chlorine solution the bark is stripped off from the woody core and the thus peeled twigs are, in the usual way (MOREL, 1948), dipped into alcohol 96 % and washed twice with sterile water.

In the case of the remaining plants the herbaceous stems were treated with the chlorine solution and the outside layers, epidermis, cortex and pericambium, scraped off. Finally, the treated stems are rinsed with alcohol 96 % and sterile water as described before.

The twigs and stems thus prepared are cut under the protection of sterile filter paper into pieces of half an inch long. With the aid of specially made and sterilizable copper borers cylinders of medullary parenchyma can be punched out. The pith cylinders thus obtained can be pushed out of the borers by means of exactly fitting copper cores. The diameter of the isolated pith cylinders depends on the quantity of pith present in the stems and the twigs of the plants used. In the case of *Sambucus* the diameter of the cylinders measured 2.5–3.0 mm., in that of *Ginkgo* it amounted only to 1.0 mm.

After isolation the cylinders are transferred into culture tubes supplied with nutrient agar. Two methods to bring the cylinders into contact with the nutrient medium have been practised, either by laying them on the surface of the medium, or by sticking one end into the agar. As to the second method in the majority of cases the top end has been stuck into the medium, in some series both basal or distal ends were put into the agar. A more or less strongly expressed polarity of the pith cylinders could be observed.

In the case of *Taxus* a different procedure had to be followed. The twigs of this tree enclose a very narrow medullary core. With a suitable borer, diameter .65 mm., the pith can be punched out but no proliferation in vitro could be obtained. It is possible that relatively too many cells become injured to allow proliferation. In this particular case proliferation of pith could be obtained by sticking pieces of twigs of about 1.5 inch long, with the bark stripped off, into nutrient agar. Pith cells from the surface proliferate in a relatively short time to form a loosely adhering subglobose mass of cells. In view of the polarity the topend of the pieces has to be stuck into the medium.

Particulars about microtechnical methods will be given in the special parts.

SUMMARY

Plant tissue cultures exhibit in the majority of cases a diversity of cell types. This may be caused either by the complexity of the explanted material or by its potencies.

Only in a few cases tissue cultures have been obtained consisting of one type of cells. These cultures were derived from cambium. For the experimental approach of the problem of plant cell differentiation these simple tissue cultures may prove to be important.

It is conceivable that a simple tissue might breed true. This consideration and the fact that it differentiates very early in stem development led us to choose pith parenchyma as a starting material. It might have lost some of its potencies and have become more or less physiologically and morphologically fixed as seems to be the case with some types of idioblasts. This, however, proved not to be the case.

It is our intention to discuss the results obtained with the plants

investigated in a series of short papers. They will concern *Helianthus tuberosus* L. *Sambucus nigra* L. var. *pendula* Dipp., *Zinnia elegans* Jacq., *Ginkgo biloba* L. and the variety *aurea* Carr. of *Taxus baccata* L.

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