CELL SEPARATION IN ISOLATED ABSCISSION ZONES

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Abstract

A technique is described for the isolation and maintenance culture of 250 μm thick slices of the leaf abscission zone of *Coleus*. Such cultures can be observed under the light microscope and a continuous photographic record of the cytological events associated with abscission can be obtained. Certain stages of the process can be accurately defined and the opportunity arises for closely correlated cytological and physiological studies by exposing the tissues to agents known to influence abscission. Some examples illustrating these points are discussed briefly.

I. INTRODUCTION

Work on the physiology and cytology of abscission has been based on two systems, namely intact plants and explants consisting of excised nodes with attached petiole stumps. Light microscope studies have led to the suggestion (Addicott and Lynch 1955) that, depending on the species, three mechanisms may be involved in the process of cell separation: (1) the middle lamella between cells may dissolve, the primary walls remaining intact; (2) both the middle lamella and the primary walls may dissolve leaving protoplasts covered only by thin cellulosic walls; and (3) entire cells of one or more layers may break down. However, cell division and differentiation may be involved in some cases, e.g. *Phaseolus*, in the sequence of cytological events that lead to separation (Webster 1970).

Much of the physiological work has been carried out with explants since they can be used under the precisely controlled conditions necessary for this work. Various hormones, synthetic growth regulators, and specific metabolic inhibitors have been used either singly or in combination in an attempt to elucidate the role of these substances in the control of abscission (e.g. Addicott 1965; Cooper *et al.* 1968; Jacobs 1968).

Although the above approaches have produced information on the cytology and possible controlling mechanisms of abscission, they do not satisfy the important requirement of precisely correlating physiological and cytological events in time. It is not possible, for instance, to apply a given treatment at a specific and precisely determined stage of abscission and follow its effect. This paper describes a method for maintaining isolated abscission zones which apparently behave like the other two systems and at the same time provide a convenient system for the continuous observation and experimental manipulation of the cell separation process in a given abscission zone so that correlations between physiological and cytological changes can be studied. No attempt is made in this paper to test any particular idea regarding the controlling mechanism of abscission.

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II. Methods

Coleus blumei was used because it has a cytologically well-defined abscission zone at the base of the petiole throughout the development of the leaf and consequently it is always possible to predict in which cells changes are likely to occur. Plants were grown from cuttings in the glasshouse and used when they had 7–8 pairs of expanded leaves.

Selected nodes were surface-sterilized on the plant by swabbing with 70% ethanol. All subsequent operations were performed under sterile conditions. The petioles were cut off about 3 mm from the base and the node excised as normally done in the preparation of explants. Longitudinal slices of the abscission zone, about 250 μm thick, were prepared and mounted in culture chambers as shown in detail in Figure 1. All experiments were carried out at 22°C.

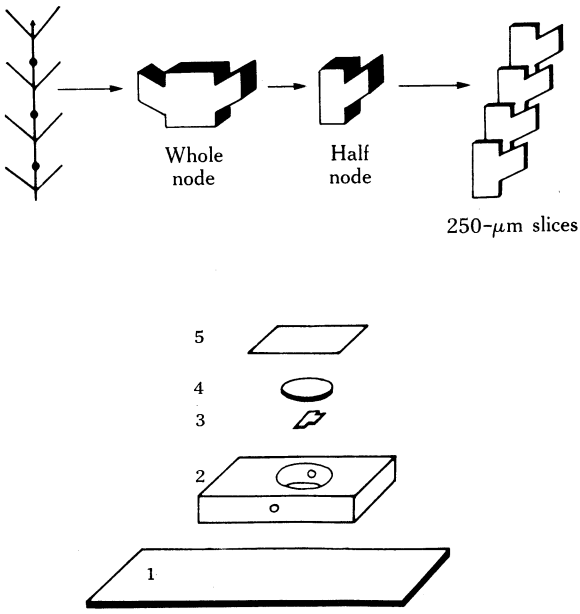

Fig. 1.—Preparation of abscission zone cultures. Four 250 μm slices were obtained from the median longitudinal region of each halved node explant by using a sliding hand microtome. In experiments involving a number of treatments slices from a given node were distributed between controls and treatments so that variations due to the origin of the material were reduced. The agar substrate (*4*) was prepared by placing about 0·5 ml of molten medium (2% sucrose plus 0·5% agar) on a coverslip (*5*) using as a mould a brass ring that was removed after the agar had gelled. The tissue slice (*3*) was then placed on the agar medium, the coverslip inverted and positioned over the hole (18 mm diameter by 5 mm depth) of the Perspex culture chamber (*2*) which was already attached to the microscope slide (*1*). All components of the chamber were previously sterilized and sealed during assembly with sterile petroleum jelly. The two holes at the sides of the chamber allow the introduction of gases or micropipettes and can be sealed with jelly or cotton wool. A condenser with a long focal distance (e.g. 7 mm) was used for observation of the culture under a Zeiss light microscope.

This system lends itself readily to continuous observation under the light microscope and allows a complete record of the events in a given abscission zone to be obtained by means of still photography or time-lapse cinematography. The process of abscission can be interrupted at any precisely defined stage and the culture prepared for further light or electron microscopy using standard techniques. The effect of various substances on the process can be tested either by incorporating them in the culture medium or applying directly on the tissue. In addition, the effect of volatile substances, e.g. ethylene, can be studied by introducing them into the chamber.

III. Observations and Discussion

One of the more valuable advantages of this method is that a continuous record of the process of abscission can be obtained as illustrated in Figure 2. The sequence of micrographs shows the progression of a group of cells in the abscission zone from the time of excision to cell separation. On the basis of such observations it has been

possible to resolve the process into a number of well-defined stages that can be assigned arbitrary numerical values as described in Figure 4. In this way a semi-quantitative representation of the time course of the process can be reconstructed.

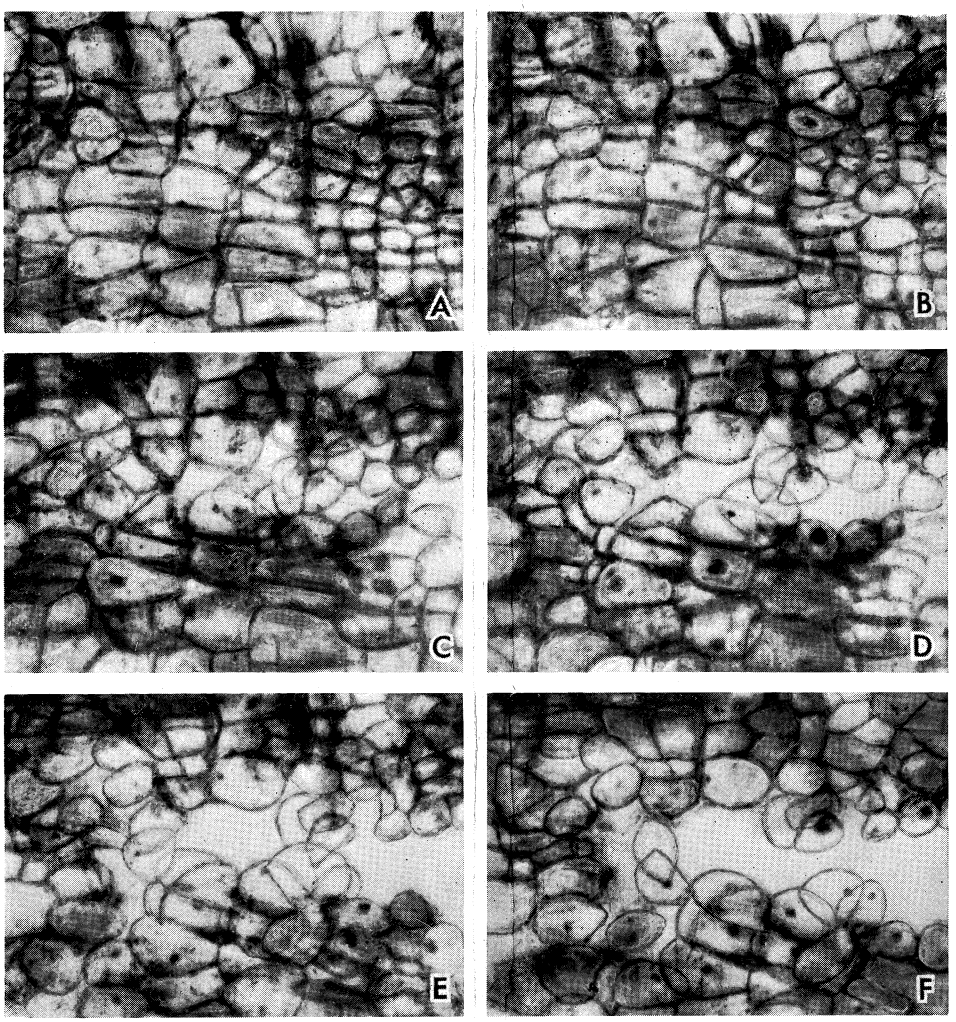

Fig. 2.—Stages, selected from a time lapse sequence, in the separation of cells in the abscission zone of a control culture. The elapsed times in hours, from setting up the culture, are as follows: *A*, 0 hr; *B*, 60 hr; *C*, 65 hr; *D*, 67 hr; *E*, 69 hr; *F*, 90 hr. The separation process starts at the ventral side of the petiole slice.

Another advantage of the method is that cytological studies can be performed at precisely defined stages of the process. For example, pectin changes in the cell walls of the abscission zone can be followed by treating cultures at defined stages of the process with alkaline hydroxylamine–ferric chloride (Jensen 1962). It was found that immediately after excision the stain was localized in the middle lamellae of the cells

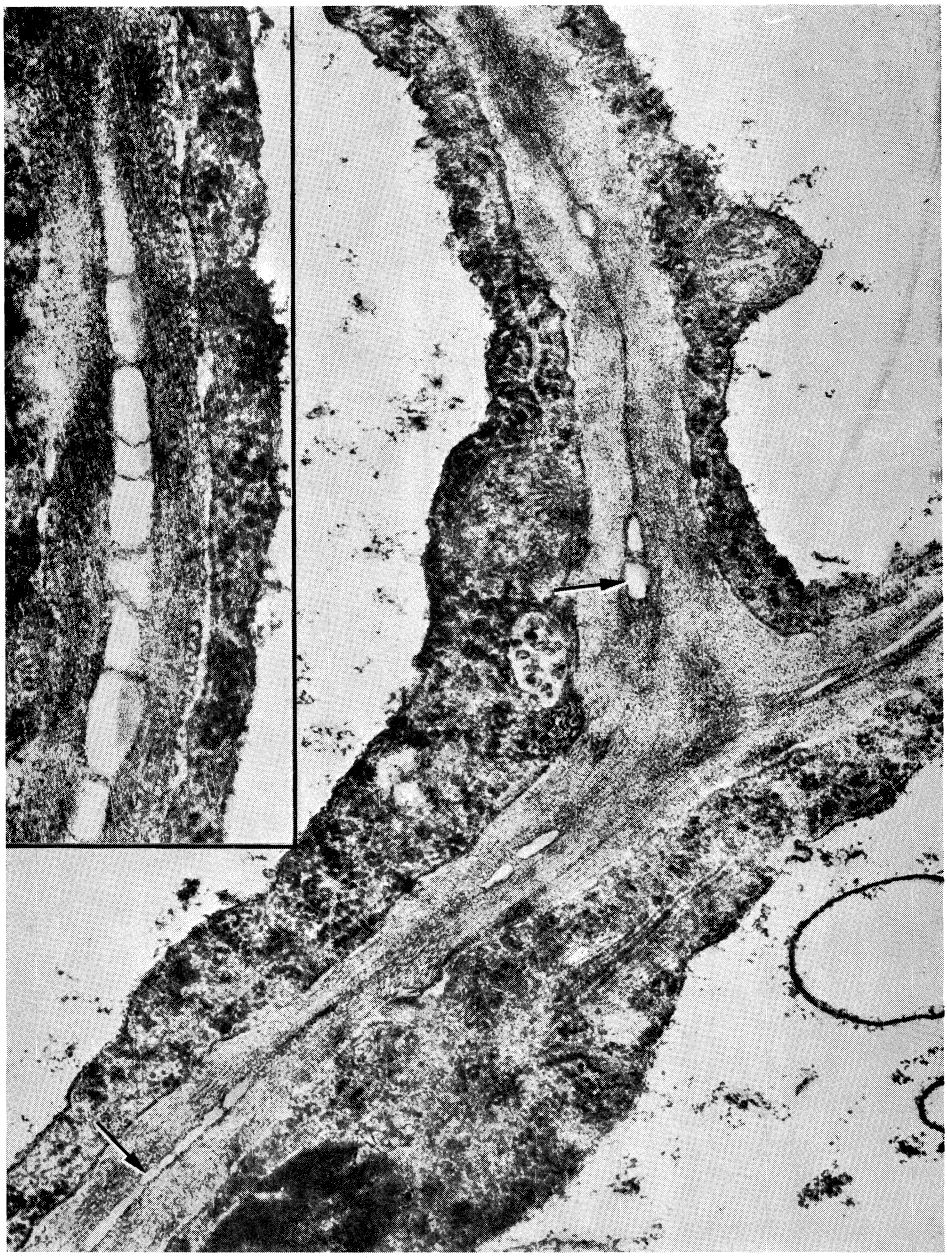

Fig. 3.—Typical appearance of the cell walls in a cultured abscission zone during the formation of the dark band (see Fig. 4, stage $-0{\cdot}5$). The arrows indicate regions where the middle lamella begins to dissolve. $\times 62{,}000$. The inset shows a higher magnification ($\times 81{,}000$) of such a region. Cytoplasmic preservation was never satisfactory although different fixation procedures were attempted. Glutaraldehyde/OsO_4 fixation.

throughout the tissue. Twenty-four hours after excision, that is at the "dark band" stage (see legend to Fig. 4), the staining in some walls of the abscission zone was discontinuous and in others completely missing. By the 48th hour very little staining was present in the walls of the abscission zone, while in contiguous regions of the tissue the staining characteristics of the walls were the same as at the time of excision. These cytochemical changes in the middle lamella are reflected in the appearance of the walls, for example during the dark band stage, under the electron microscope (Fig. 3). These ultrastructural changes in the cell walls are identical with those reported by Bornman (1967) for *Coleus* explants.

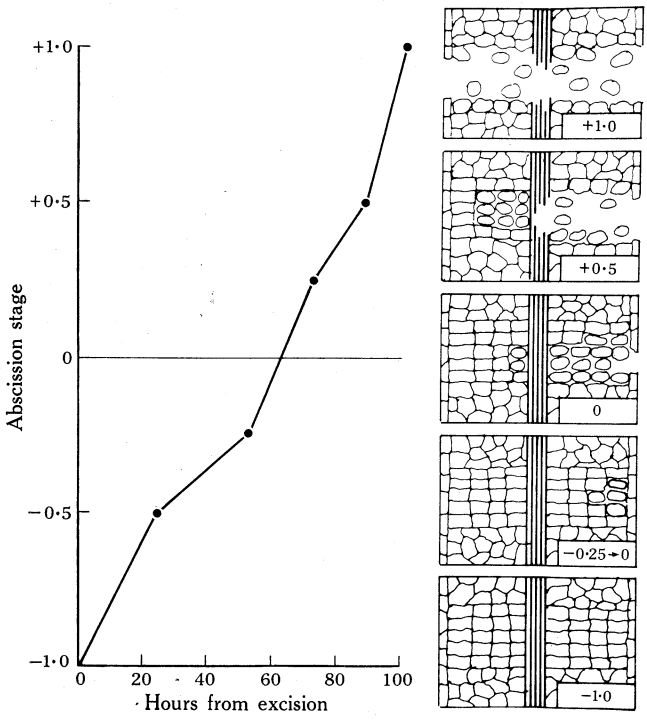

Fig. 4.—A typical time course of cell separation in an abscission zone culture and definition of abscission stages. Since it is easy to determine if an abscission zone is a quarter, half, three-quarters, or completely separated the stages of separation can be given corresponding positive values ranging from 0 (many cells rounded, separation about to begin) to +1·0 (separation completed). The period preceding the beginning of separation can be described by ascribing negative values to recognized stages. The value −1·0 is given to the culture immediately after excision. Since the first recognizable change is a transient increase in the opacity of the cytoplasm of the abscission zone cells that results in the gradual formation of a distinctive "dark band" in the abscission zone, a culture with a faint dark band is given the value of −0·5 that progresses to −0·25 as the band gets more dense. Values between −0·25 and 0 refer to progressive stages in the rounding up of the cells. The diagrams to the right of the graph give some idea of the standards used in scoring cultures. The numbers with each diagram identify the stage represented. The abscission zone is represented by the band of rectangular cells and the vascular tissue by the vertical lines. The plotted points are based on the results obtained with a particular control culture.

The method described here provides the opportunity to correlate cytological behaviour with the physiology of the process. Figure 5 illustrates some typical data that have been obtained on the effect of indoleacetic acid, when applied in the medium, on the rate of abscission. It is seen that the inhibiting effect of auxin on the process of abscission is similar to that normally obtained with intact plants or explants. Experiments, not reported here, with other growth regulators (gibberellic acid,

kinetin, abscisic acid) and protein synthesis inhibitors (cyclohexamide) indicated that the behaviour of excised abscission zones is generally comparable to that of whole node explants as described in a number of recent reports (e.g. Addicott 1965; Abeles 1968). In view of the importance that the results of proximal or distal applications of auxin to explants have had on the ideas regarding the hormonal control of abscission it should be mentioned that similar tests can be made on tissue slices. Blocks of agar containing the substance to be tested can be placed on top of the tissue slice and in the proximal or distal side of the abscission zone. However, the mode of transport may be different in thin tissue slices when compared with explants.

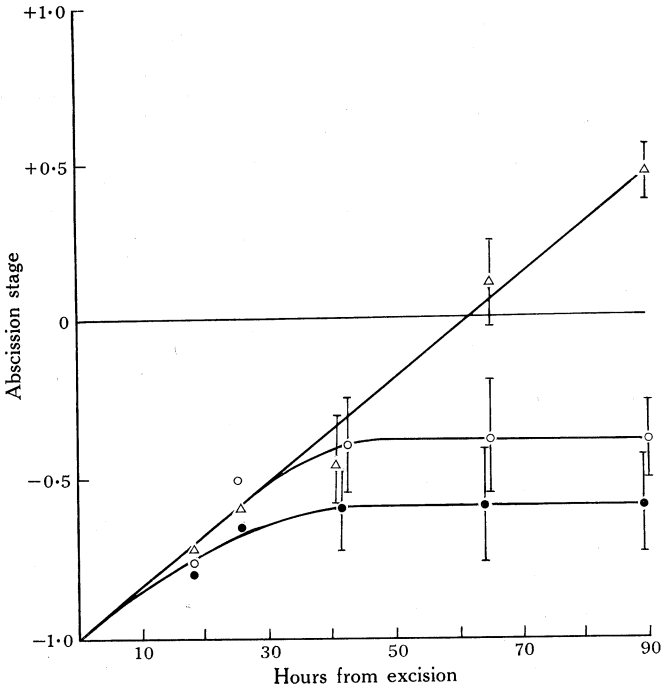

Fig. 5.—Effect of indoleacetic acid (IAA), incorporated in the medium before preparation of the culture, on the rate of cell separation. Each point represents the mean abscission stage attained at a particular time in three different experiments with five replicates per treatment per experiment. Standard errors based on the 15 readings per point are shown by the vertical lines. In view of the closeness of the points early in the experiment the standard errors are not shown. △ Control. ○ 1 p.p.m. IAA ● 10 p.p.m. IAA.

In some experiments even control cultures may fail to separate completely unless they have done so by about the 100th hour. Apparently the present culture conditions are not suitable for maintaining the tissue in a healthy condition beyond that time. Complete nutrient media [e.g. White's (1963)] have been tried with no improvement in the life span of the culture.

In some ways the type of semiquantitative measurements that can be obtained at the cellular level with this method is comparable to the quantitative determination of changes in the break strength of the abscission zone (Morre 1968). The main difference is that while the method of break strength analysis provides a more reliable quantitative study of the state of the abscission zone as a whole it does not yield information at the individual cell level. Also, in break strength measurements, different explants are used for each determination, whereas in the method described here the same culture, or even a group of cells, can be followed continuously and regional differences in behaviour over the abscission zone can be defined.

In spite of the obvious advantages of this method in studying the process at the cellular level it is equally obvious that the problem of extrapolating to the intact plant is as serious here as in the case of explants. Although the cytological changes which occur during abscission in the different systems may be the same, neither cultures nor explants will ever simulate the complex interactions of hormonal and nutritional factors in the intact plant. It is clear that an integrated approach, involving the three systems—intact plants, explants, and cultures—should be the most profitable method of investigating the phenomenon of abscission.

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