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FINE STRUCTURE OF THE ABSCISSION LAYER IN PHASEOLUS FLOWERS UNDER WATER STRESS CONDITIONS

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SUMMARY

It has been observed that under control conditions, the abscission of flowers in *Phaseolus vulgaris* proceeds via the breakdown of both the middle lamella and the primary wall of the cells in the abscission layer of the pedicel. However, under conditions of water stress, the dissolution of the middle lamella occurs much faster than the breakdown of the primary wall. The difference in the integrity of the cell wall of the abscising cells under these two conditions is discussed in relation to differential enzyme activation of the separation process.

1. INTRODUCTION

The abscission of a plant organ occurs in a special layer, the abscission layer and is achieved either by dissolution of the middle lamella between two layers of cells thus leaving the primary wall intact or by dissolution of both the middle lamella and the primary wall, whereas in other cases whole cells are dissolved (SCOTT et al. 1948; VALDOVINOS & JENSEN 1968; VALDOVINOS et al. 1972; SEXTON & HALL 1974). Most of this information has been gained from ultrastructural studies of foliar abscission.

So far, only JENSEN & VALDOVINOS (1967) and VALDOVINOS & JENSEN (1968) have attempted to show the ultrastructural pattern of the abscission process in flowers. These workers, while looking at the fine structure of abscission zones in the abscising pedicels of tobacco and tomato flowers, observed that prior to the initial stages of cell wall disintegration, there is a structural change of microbodies with crystalloid cores. The microbodies tend to be very prominent. They suggested that one possible function of the microbodies was to act as storage sites for enzymes which bring about lysis of entire cells – which was actually observed during the course of the abscission of the flowers in question. It is thus evident from this work that the abscission of flowers, at least of these two species, tends to favour the dissolution of whole cells as they abscise.

The present study attempts to throw more light on the ultrastructural changes occurring in the abscission layer of the flower pedicels as they abscise, particularly under comparative conditions of water stress.

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2. MATERIALS AND METHODS

Plant Materials: Seeds of *Phaseolus vulgaris* L. (local variety coded as UAC cw. 121) obtained from Uyole Agricultural Research Station Mbeya, were sown directly into 7 inch pots containing garden soil made up of yard manure and soil (3:1 v/v). After germination, the plants were thinned to one per pot and they were watered to field capacity daily. When flower buds started to form, the plants were divided into two sets, one set was watered daily while the other set was subjected to water stress by withholding water for intervals of up to six days.

Preparation of the pedicel abscission zone tissue for electron microscopy: Sections from abscission zones at anthesis and four days after were cut and fixed overnight in 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.4) at 0°C and subsequently washed overnight in sodium cacodylate buffer (0.05 M) containing 0.25 M sucrose in cold conditions (± 0°C). The material was then post-fixed in 1% osmium tetroxide in 0.3 M phosphate buffer at 0°C for two hours and rinsed several times in distilled water; followed by dehydration using tertiary butyl alcohol series. Dehydration was followed by clearing with propylene oxide at room temperature for 30 minutes. The material was then embedded in a series of propylene oxide and TAAB. Sections of 60–90 nm were then cut using an ultra-microtome, taken up in copper grids of about 300 meshes per sq. inch and stained in uranyl acetate solution for 10 minutes. The sections were observed and photographed on a Zeiss E.M. 9 s-z electron microscope.

3. RESULTS

The characteristic thin-walled cells of the abscission zone in the pedicel of non water-stressed flowers are shown in *fig. 1* particularly in cells "a" and "b". At anthesis, symptoms of wall separation are not yet visible in the tissue.

Fig 2 illustrates cells of the abscission zone, four days after anthesis in daily watered plants. At this stage, the cells of the abscission zone are larger and the process of abscission has already started. The expected symptoms of separation such as the presence of prominent rough endoplasmic reticulum (RER), plasma membrane separation from the cell wall and identifiable areas of future breaks, have become clearly visible.

Fig. 3 is an enlarged view of the cells undergoing separation. The separation of the plasma membrane is seen more clearly as well as indications of invaginations of the plasma membrane into the cytoplasm.

Fig. 4 shows a plasma membrane which has almost completely separated from the cell wall and the invaginations are very prominent. In addition, some areas of the middle lamella have become more transparent, an indication of the starting of the dissolution process in this part of the wall.

Figs. 5 and *6* show cells from the abscission zone of flowers at anthesis from a water stressed plant. The walls are particularly thin and the cells are crumpled. The plasma membrane has already separated from the cell wall and well devel-

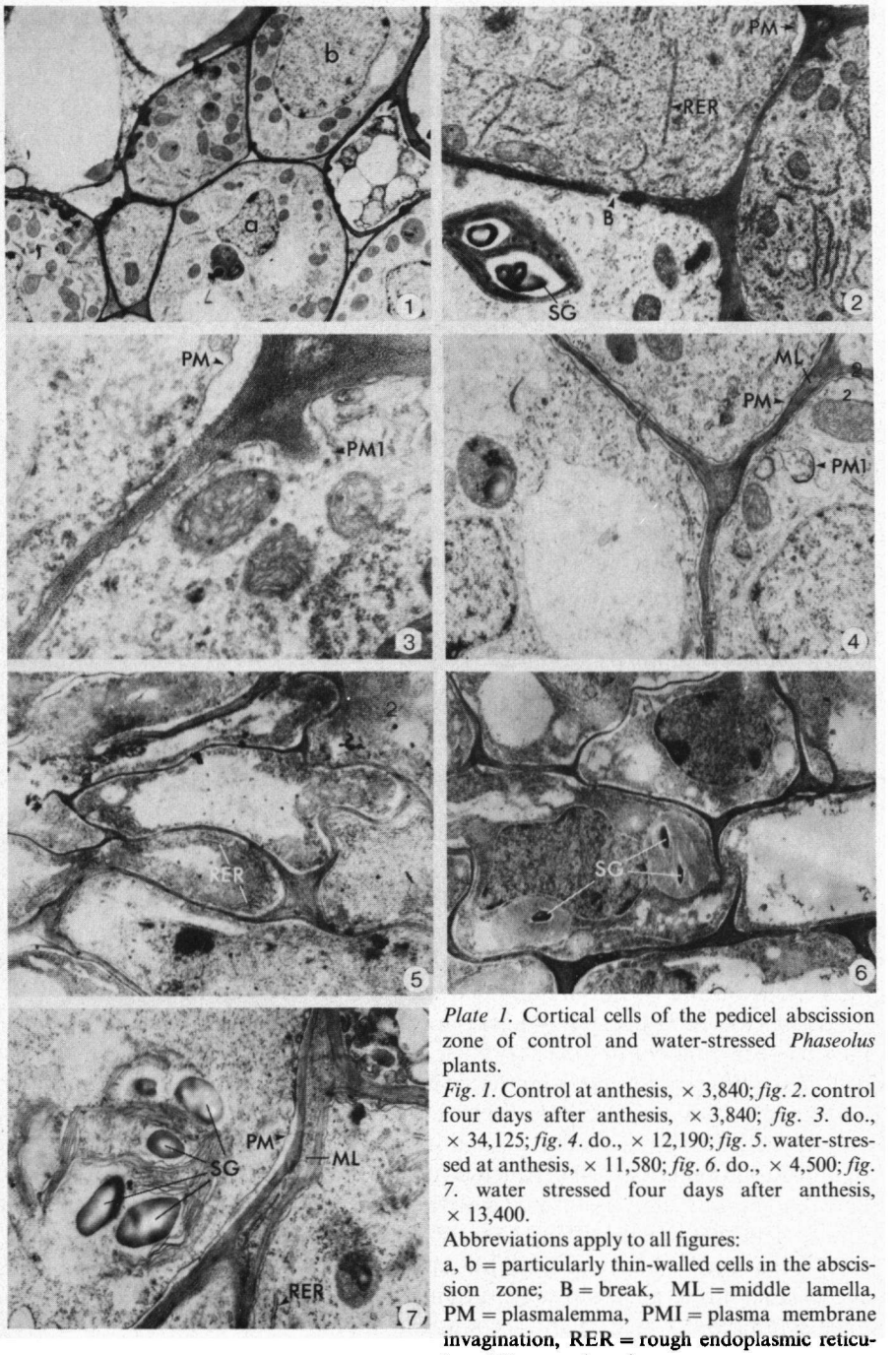

Plate 1. Cortical cells of the pedicel abscission zone of control and water-stressed *Phaseolus* plants.

Fig. 1. Control at anthesis, × 3,840; *fig. 2.* control four days after anthesis, × 3,840; *fig. 3.* do., × 34,125; *fig. 4.* do., × 12,190; *fig. 5.* water-stressed at anthesis, × 11,580; *fig. 6.* do., × 4,500; *fig. 7.* water stressed four days after anthesis, × 13,400.

Abbreviations apply to all figures:

a, b = particularly thin-walled cells in the abscission zone; B = break, ML = middle lamella, PM = plasmalemma, PMI = plasma membrane invagination, RER = rough endoplasmic reticulum, SG = starch grains.

oped starch grains can be seen. In addition, rough endoplasmic reticuli are clearly visible. All these are signs of marked development towards abscission.

Finally, *fig. 7* is an enlarged view of cells from the abscission zone of flowers from water stressed plants taken at four days after anthesis. It is shown here that the dissolution of the middle lamella is quite advanced and that the walls are almost completely separated. Notable also are the prominent starch grains.

4. DISCUSSION

Observations based on the flowers of control plants (*figs. 1–4*) indicate that in *Phaseolus vulgaris* L., the separation process seems to be accomplished by the participation of both the middle lamella and the primary walls. The involvement of the primary wall is evident in *figs. 2* and *4* where indications of breakage points are seen in the main wall. However, in the same *figs. 2* and *4* it can be seen that the middle lamella shows signs of dissolving before there is a total cell-wall breakdown, an indication that it also plays a role in the overall process of separation. These results are, therefore, in agreement with the findings of WEBSTER (1973) who, while studying the abscission of leaves in intact plants of *Phaseolus*, observed that cortical cell separation involves breakdown of both the middle lamella and the primary wall. She observed that three major cell wall changes are apparent: dissolution of pectic substances and consequent breakdown of the middle lamella, reduction of insoluble polysaccharides in the primary wall, and occurrence of actual breaks in the longitudinal and radial walls of cells.

On the other hand, *figs. 5–7* of flowers of water-stressed plants show that with water stress, the dissolution of the middle lamella occurs much faster than the breakdown of the primary wall. This is particularly clear in *fig. 7* where the cells are almost completely separated while the breakdown of the primary wall is not yet very pronounced. This finding is closer to the report of SEXTON & HALL (1974) who observed that separation in bean leaves occurred primarily as a result of the dissolution of the middle lamella region of the walls, leaving intact cells on the two newly exposed fracture surfaces. In other words, both in the SEXTON & HALL's (1974) case and in our present study, the separation may be occurring before there is much conformational change in the primary wall. This difference in the integrity of the primary wall of flowers from plants under control conditions compared to that of flowers from water-stressed plants may be interesting, particularly when it is seen in the light of there being no significant difference in cell organelles such as the rough endoplasmic reticulum known to be intimately associated with the process of abscission. It cannot therefore be said that it is the ability to synthesise cell wall degrading enzymes, normally associated with rough endoplasmic reticulum which explains this difference, rather it is tempting to suggest that it is the level of activity of the enzymes once synthesized which might account for this observed difference. It is presumed here that water stress conditions cause not only an acceleration of the

separation process but also alters the pattern of separation making the one dependent on the dissolution of the middle lamella more pronounced; and that this is done through the differential activating effect of water stress on the level of activity of the cell wall degrading enzymes favouring more those which are involved in the dissolution of the middle lamella.

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