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The Development of Structural Changes in Epidermal Cells of Maize Roots During Water Stress

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Abstract. Ultrastructural changes following increasing periods of water stress induced by means of polyethylene glycol 4000 (from 5 min to 18 h) were investigated in young epidermal cells of the primary roots of *Zea mays*. The sensitivity of the individual cell components to water stress was considered according to the time sequence in which their alterations appeared.

Endoplasmic reticulum (ER) and mitochondria proved to be the most sensitive cell components, their structure changing after 5 min of water stress. By 8 h of stress, the condensation of nuclear chromatin in some cells was apparent, preceding polyribosome degradation but not all the other changes of the stressed cells. Similar types of structural alterations appeared slightly earlier in the more differentiated epidermal cells. The rapid changes in the structure of ER and mitochondria coincided with the rapid decrease of water potential of the root tips and the immediate cessation of the root growth.

Almost any parameter or process of the plant can be changed by water stress that is severe and long enough (HSIAO and ACEVEDO 1974). The sensitivity of individual processes to water stress varies (HSIAO 1973, HSIAO and ACEVEDO 1974, HSIAO *et al.* 1976). The most sensitive appears to be cell growth that immediately responds to the decrease of cell turgor. The inhibition of growth is accompanied by inhibition of cell wall synthesis. Protein synthesis is also very sensitive to water stress but the synthesis of some proteins is reduced by water stress while that of others is intensified (BEWLEY *et al.* 1983). When stress is severe enough, further metabolic processes are suppressed (BRADFORD and HSIAO 1982).

Similarities in the response pattern to water deficit may also be found at the ultrastructural level. The structure of almost all the cell organelles is changed if the water stress is severe enough (NIR *et al.* 1969, VIEIRA DA SILVA 1976), but little attention has been given to the studies of the differential sensitivity of cell organelles. The aggregation of nuclear chromatin and displacement of lipids from various cellular membranes are suggested to be the key events preceding all other changes and damage induced by water stress (POLJAKOFF-MAYBER 1981).

After 24 h of water stress induced by polyethylene glycol 4000 conspicuous damage of the epidermal cells of maize roots occurred although the inhibition

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of root growth was reversible (ČIAMPOROVÁ and LUXOVÁ 1976, ČIAMPOROVÁ 1976, 1977). Ultrastructure of these cells after the increasing periods of water stress has been investigated to find out the time sequence of the ultrastructural alterations of the individual cell components. The differential sensitivity of cell organelles was considered in the light of the appearance of their alterations.

MATERIALS AND METHODS

Seeds of maize, *Zea mays*, inbred line A15 (VIR 17) were germinated on moistened filter paper for 48 h at 25 °C, in the dark and the seedlings allowed to grow in 1/2 Hoagland nutrient solution (osmotic potential π -0.037 MPa) for 24 h before transfer to a solution of 35 g PEG 4000 (polyethylene glycol) in 1000 ml of the same nutrient solution (π -1.56 MPa), where they were exposed to water stress for 5, 10 and 20 min,1, 2, 4, 8, 12, 16 and 18 h. The decrease of water potential of the root tips and the growth inhibition of the primary roots have been reported recently (ČIAMPOROVÁ and ELIÁŠ 1987). Control plants were grown in 1/2 Hoagland solution. For electron microscopy, routine techniques of fixation in glutaraldehyde and OsO_4, dehydration in ethanol series and embedding in Epon or Spurr's medium were used as previously (ČIAMPOROVÁ 1977). Ultrathin sections (stained with uranyl acetate and lead citrate) of the young meristematic cells, and more differentiated cells of the root epidermis at distances of 0.5, 1, and 2 mm from the cap junction were investigated with a Tesla BS 500 electron microscope.

RESULTS

The submicroscopic structure of the control root epidermis did not differ from that observed earlier (ČIAMPOROVÁ 1976, 1977).

The sequence of the structural changes of the individual organelles of maize root epidermal cells is summarized in Table 1. After as little as 5 min of water stress, elongation and parallel arrangement of ER elements can be observed (Figs. 1 and 2). This alteration is particularly conspicuous in the meristematic cells which in control conditions contain short, poorly developed ER elements. Mitochondria also reveal considerable sensitivity to water stress: structural responses including swelling, reduction of cristae (Fig. 3) and alteration to dumb-bell-like or irregularly elongated shapes (Figs. 4 and 5). The swelling and the reduced cristae are the earliest structural changes occurring first in the more vacuolated epidermal cells (Fig. 3). In these cells, the cristae reduction is more conspicuous since the more differentiated cells have mitochondria with more extensive cristae in control conditions. Alteration of the mitochondrial shape appears in the younger epidermal cells earlier than in the more differentiated ones. Following the longer periods of water stress, the number of mitochondria in a cell section decreases. Distorted mitochondrial profiles occur occasionally inside vacuoles where their disintegration apparently occurs. Undamaged mitochondria are still present (Fig. 6) in addition to mitochondria showing modified structure or mitochondria with a dense matrix, following 18 h of stress. The outer mitochond-

TABLE 1

The sequence of structural alterations of endoplasmic reticulum (ER), mitochondria (M), dictyosomes (d), nucleus (N), ribosomes (r) and polyribosomes (pr) in the stressed root epidermal cells. The occurrence of the alterations is not repeated; only addition of new types of alterations is recorded

Stress duration	Distance of epidermal cells from root cap junction [mm]		
	0.5	1.0	2.0
5 min	ER – elongated		M – swelled
10 min	ER – parallel arrangement	ER – parallel arrangement	ER – elongated
20 min	M – swelled – reduced cristae	M – changed shape	
1 h	M – changed shape	d – incompact	d – incompact
2 h		M – swelled d – reduced vesicle production	M – changed shape d – reduced vesicle production
4 h	d – incompact – reduced vesicle production		
8 h			N – condensed chromatin pr – less frequent
12 h	r – less frequent	d – disintegrated r, pr – less frequent	d – disintegrated
16	M – less frequent d – disintegrated	M – less frequent	M – less frequent
18 h	M – dense matrix N – condensed chromatin	M – dense matrix N – condensed chromatin	M – dense matrix

rial membranes remain continuous. Dictyosomes are not affected and produce large and numerous vesicles following short periods of stress (Fig. 7). Less compact dictyosomes with a higher number of cisternae producing less numerous and smaller vesicles occur in the older epidermal cells as well as in the meristematic cells following 1 and 4 h of water stress, respectively (Figs. 8 and 9). Disintegration of the dictyosomes continues when the stress is prolonged (Figs. 9 and 10). The lower density of ribosomes in the cytosol can be observed in the sections of cells stressed for 12 h (Fig. 11). The cytosol appears to be markedly clear of organelles when compared to cells of the same age after the first few minutes of water stress (Figs. 1 to 4). In the older, more differentiated cells, numerous polyribosomes are present during the shorter periods of stress, while they are very few after 12 h of stress (Fig. 10). The nuclear membrane remains continuous (Fig. 12) and nuclear chromatin condensation (Fig. 13) can be observed in some cells after 8 h of water stress but even after 18 h of stress this structural response does not occur in every cell. The structure of plastids remained unchanged. Microtubules were present in the younger cells throughout all the stress periods but were not observed in the more differentiated epidermal cells stressed for longer than 8 h.

The structural alterations observed in the root epidermal cells stressed at three different stages of differentiation are of the same type. They usually appear earlier in the more vacuolated cells than in the younger meristematic cells. There is also some variability in the structural response between epidermal cells of the same age. Two neighbouring cells in the transverse section may differ markedly in the density of their cytosol even in the intact roots (Fig. 14). These differences in the ultrastructure and its response to water stress might arise from the fact that the cells occur in different phases of the cell cycle.

DISCUSSION

The epidermal cells of the root are in direct contact with the environment inducing the water deficit. This enables relatively rapid structural response of the cell components to occur. The readiness of the individual cell components to alter their structure determines their sensitivity to water stress and the most sensitive appear to be the ER and the mitochondria. The rapid elongation of ER and subsequent elaboration of parallel arrangements correspond to the high sensitivity of protein synthesis to water deficit (Hsiao and Acevedo 1974) and the changed pattern of protein synthesis in the stressed plants (Bewley *et al.* 1983). Water stress also lowers the activity of some enzymes while the activities of others increase (Luna *et al.* 1985). The increased elaboration of the ER system might also result from decreased utilization of the ER cisternae to form the cisternae of dictyosomes similarly to that occurring under low temperature stress (Mollenhauer *et al.* 1975).

The rapid response of the mitochondrial structure to water stress might be associated with the immediate decrease of water potential in the root tips and cessation of growth of maize seedling roots (Čiamporová and Eliáš 1987). The reduction of mitochondrial cristae may accompany the decrease of respiration that occurs when root growth and protein synthesis are inhibited and the demands for respiratory energy decline (Pheolung and Barlow 1981, Thomas and André 1982). The dumb-bell shaped mitochondria may represent the division of the organelle and thus an adaptation response to water stress. Although fewer mitochondria are observed in the cell sections after prolonged dehydration, there are still some organelles with well developed internal structure even after 24 h of stress which would enable the regeneration of root growth after rehydration (Čiamporová 1980).

The inhibition of root growth may also be primary to the structural changes of dictyosomes. In the intensively elongating older cells, the disintegration of dictyosomes therefore occurs earlier than in the younger meristematic cells.

The occurrence of the condensation of nuclear chromatin in various tissues and plant species and its possible effects on the transcription and translation processes in the stressed cells were considered in suggesting this response to be one of the central events which precede all other changes and damage induced by water stress (Poljakoff-Mayber 1981). This structural response may precede the destruction of ribosomes thus inhibiting protein synthesis when the stress is prolonged. However, if the structural alterations of endoplasmic reticulum, mitochondria and dictyosomes occur prior to the aggregation of chromatin in the nuclei, the primary effects of water stress on the cell membranes rather than on the nuclear chromatin seem to be operative. The structural changes observed indicate that the combination of interference with the energy supply and delay in normal RNA turnover can bring about many of the secondary features of damage brought about in plants by water stress as suggested by POLJAKOFF-MAYBER (1981).

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