ULTRASTRUCTURAL ASSESSMENT OF THE TOLERANCE OF *NICOTIANA TABACUM* L. (Cv. Xanthi) REGENERANTS CELLS TO SUCCESSIVE STRESS INDUCED BY PEG (6000)

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The present study reveals the ultrastructural changes in cells of tobacco regenerants incubated for 45 days on the regeneration medium supplemented with 5% PEG (6000) and subcultivated during 90 days on regeneration medium supplemented with PEG (6000), in two different concentrations: 5% and 10%. The observed ultrastructural aspects confirmed that the successive treatments with PEG induced the decrease of the tolerance level of cells against the stress caused by drought, leading to the reduction of the morphogenesis potential.

Key words: PEG-polyethyleneglycol, MET-regeneration medium, tobacco regenerants, stress tolerance.

INTRODUCTION

The plant we are referring to, generally called Venus's flytrap, because of its rapidity of closing the two foliar lobes which form the trap, is one of the most interesting in the world. *Dionaea muscipula* lives in the peat bogs of North and South Carolina (S.U.A.). It is a perennial plant, having a basal rosette of leaves, from the middle of which the floriferous stem rises up.

The abiotic stress factors have special effects on live organisms, but they have generally similar effects on tissue water content. Galinski (7) realized biochemical studies that revealed certain similarities in the stress-induced processes, like the secondary metabolites accumulation.

The addition of the 5% PEG 6000 (PEG: Poly Ethylene Glycol) in the culture medium induced a decrease of the water potential into the culture medium. The changes in water potential in the culture medium lead to an osmotic stress in the plant cell that is perceived by the cell as for example plasmalemma perturbations caused by the loss in turgor pressure (12).

The drought represents a stress factor, which reduces the hydric potential, induces the decrease of cellular turgor and the increase of the cell wall elasticity (9, 13). Thus, the cell volumes become significantly reduced. Carbohydrate accumulations occur in the cells, as a response to the water shortage and as a

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tolerance against stress of this type. The carbon deposits are involved in the passive osmotic regulation, maintaining the cell turgor and preventing the cells volumes from decrease beyond certain limits.

The aim of this study is to reveal the ultrastructure modifications induced by the successive treatments with PEG, at the level of tobacco regenerant cells.

MATERIALS AND METHODS

The explants of *Nicotiana tabacum* L. (cv. Xanthi) petiole were inoculated on the regeneration medium (MET-control medium), a modified variant of the culture medium Murashige/Skoog (10); supplemented with auxine (alpha naphthyl acetic acid ANA 0,1 mg/l) and cytochinine (Benzyl-amino-purine: BAP 1mg/l).

In the second stage after 45 days, the drought stress was simulated by supplementing with 5% PEG (as inductor) the MET for the regenerant subcultivation (6).

In a later stage, after other 45 days, the regenerants were again subcultivated on another regeneration medium, supplemented or not with PEG.

In this paper, the following evidence was investigated:

a. the tolerance evidence of the regenerants obtained on MET and subsequently subjected to 5% or 10% PEG stress.

b. the tolerance evidence of the regenerants subjected to successive stress on MET culture medium supplemented with 5% PEG and subsequently on MET + 5% or 10% PEG.

The experimental scheme and the treatment variants are presented in Table 1.

The ultrastructural assessment was based on cell samples obtained by the electron microscope standard technique using Reynolds coloration (11) and visualized in Tesla BS 500 microscope.

Table 1

The experimental scheme for studying the tobacco cells tolerance to the PEG subsequent stress

Stage I	Stage II	Stage III
MET	MET	MET
		MET + 5% PEG
		MET + 10% PEG
	MET + 5% PEG	MET
		MET + 5% PEG
		MET + 10% PEG

RESULTS AND DISCUSSION

THE TOLERANCE EVIDENCE OF THE CELLS REGENERANTS OBTAINED ON MET CULTURE MEDIUM AND SUBCULTIVATED ON MET SUPPLEMENTED WITH 5% OR 10% PEG

The cell ultrastructure in the regenerants, obtained on MET culture medium and subcultivated subsequently on the same MET culture medium, showed senescence in an advanced extent. The cell nuclei of spherical shape had a granular–fibrillar chromatin and an envelope without dilatations (Fig. 1). The reduced hyaloplasm contained numerous membrane remnants, poorly organized plastids that contain in their stroma numerous plastoglobules (Figs. 2, 3). The cell wall was sinuous and the plasmalemma changes resulted in plasmolysis.

The cells of tobacco regenerants obtained on the MET culture medium and subcultivated on MET culture medium supplemented with 5% PEG revealed lobate nuclei with envelope dilatations and rarefied chromatin (Fig. 4). The hyaloplasm, here and there lysed, contained membrane remnants, secondary metabolites, atypical mitochondria, peroxisomes, as well as fusiform plastid structurally organized. The plastids had around 15 grana/chloroplast and 3–5 thylakoids/grana. The presence of lipid droplets was also observed (Figs. 5, 6, 7).

At the level of cells of *Nicotiana tabacum* L. (cv. Xanthi) regenerants obtained on the MET culture medium and subcultivated on the same culture medium supplemented with 10% PEG, it was obvious that the PEG concentration increase caused the enhancement of the alterations characteristic of the osmotic stress such as the hyaloplasm reduction and vacuolization spreading (Fig. 8). The vacuoles contained opaque to electrons deposits, possibly secondary metabolites (Fig. 9). The mitochondria were the most affected organelles while the plastids had an internal thylakoid structure. The centrally positioned nucleus was surrounded by a thin hyaloplasm film (Fig. 10). The dots formation was observed at the level of cell walls (Fig. 11).

The tolerance evidence of the regenerants subjected to successive stress: on MET culture medium supplemented with 5% PEG and subsequently on MET culture medium supplemented with 5% or 10% PEG.

The regenerants subjected to successive PEG stress presented at subcellular level certain differences against the non stressed regenerants.

In the cells of regenerants obtained on MET culture medium, supplemented with 5% PEG and subcultivated on MET culture medium, a rarefied and lysed hyaloplasm, an extended vacuolization and secondary metabolites were observed (Figs. 12, 13). The plastids were the most affected organelles (Figs. 14, 15).

The cells ultrastructure of regenerants on MET culture medium supplemented with 5% PEG, and subcultivated on the same culture medium, revealed that the 5% PEG applied in successive treatments produced more severe cellular stress,

resulting in an advanced damage of organelles structure. The mitochondria were the most affected organelles (Fig. 16). Big vacuoles containing large, opaque to electrons deposits were present (Fig. 17).

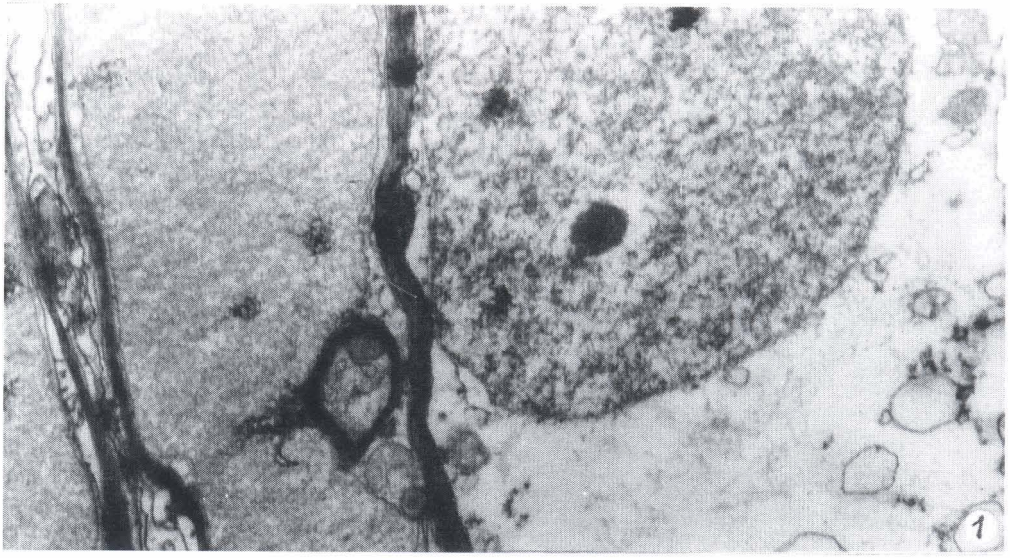
In the case of the regenerants obtained on MET culture medium supplemented with 5% PEG and subcultivated on MET culture medium supplemented with 10% PEG, it was shown that increasing the PEG concentration caused a severe lysis of the hyaloplasm and organelles within it (Fig. 18). Opaque to electrons formations, adjacent to the plasmalemma, were revealed in a large number (Fig. 19). The plastids, without an internal organized thylakoid system, contained a large number of plastoglobules, amyliferous inclusions, peripheral reticule as well as polyphenol accumulations. The polyphenol accumulations were also found in the meatus (Fig. 20). The nucleus with dilated envelope presented a tendency of euchromatization (Fig. 21).

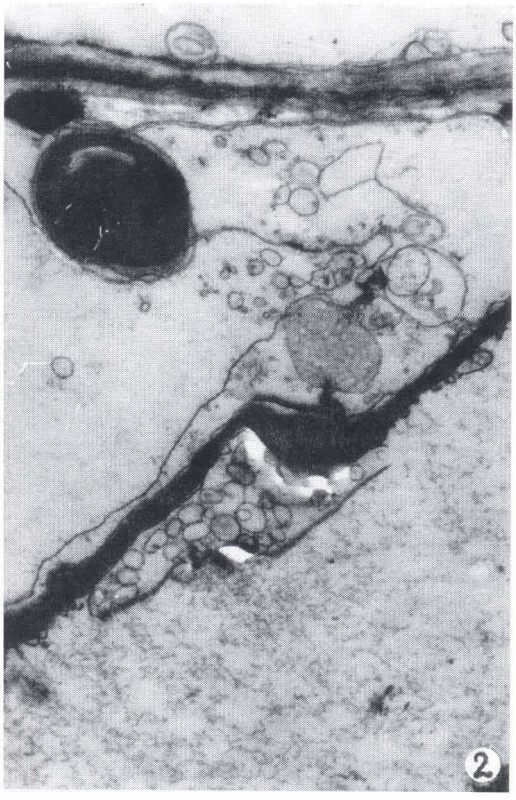
The literature data revealed the existence of certain differences between the natural tolerance to the stress and sensibilities of plants subjected to severe stress, which induces severe cell changes, the plant being forced to adapt its metabolism (5, 6). The biochemical changes caused by the water shortage provoke the alteration of stomata conductance, photosynthesis and also perturbations of ionic balance. The histological studies, carried out by Chartzoulokis *et al.* (3, 4) on olive leaves, showed that the stress factors used in their study determined a photosynthesis and transpiration diminution against control, by the stomata closing. The leaf anatomical changes consist generally in a significant increase of the cell density in the epidermis and mesophyll, as well as the intercellular space reduction. Maron (8) following his ultrastructural observations considered that the cell number increase after stress represented a contribution to turges maintaining, through the decrease of the intercellular space, which impeded the water evaporation.

Bohnert (1, 2) stated that different tolerant mechanisms to drought imply an efficient increase of the water use. PEG used by us as inductor of drought stress, enhanced the biochemical processes in cells, enabling the appearance of phenomena characteristic for the osmotic stress. The observed and above presented ultrastructural differences confirm that the successive treatments with 5% PEG and 10% PEG caused a diminution of the adaptation extent of tobacco regenerants to drought stress, which explained the reduction of their potential of morphogenesis.

CONCLUSIONS

By studying the ultrastructure modifications induced by successive treatments with PEG, at the level of tobacco regenerants cells, the following conclusions may be revealed:





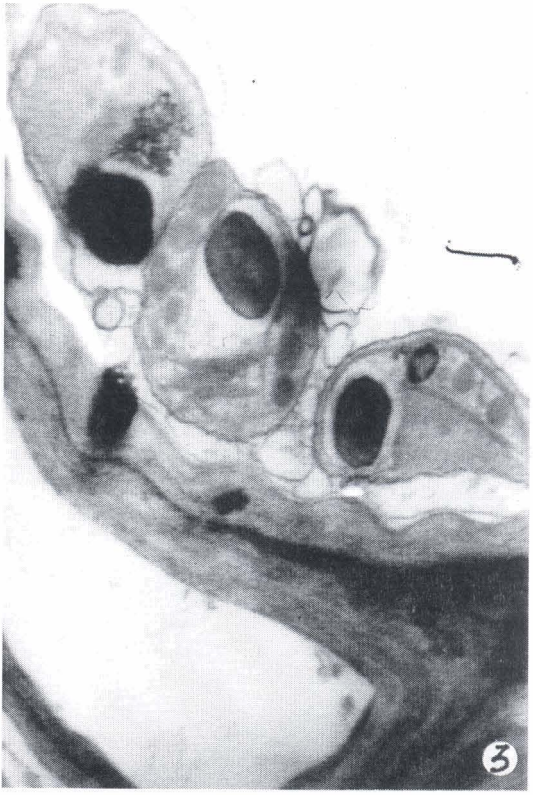
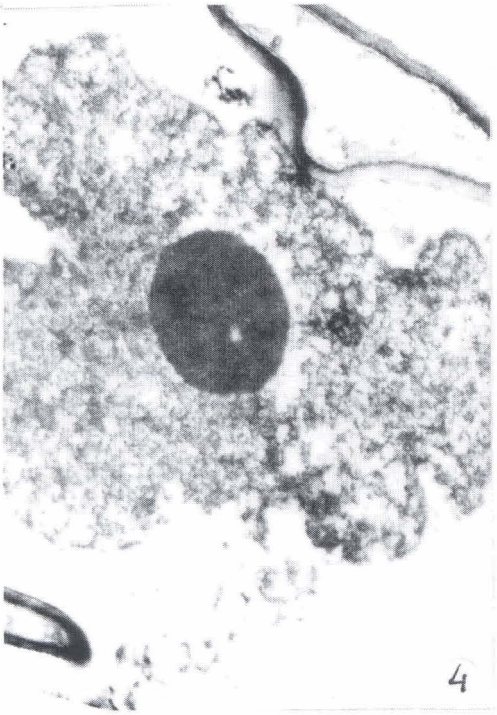
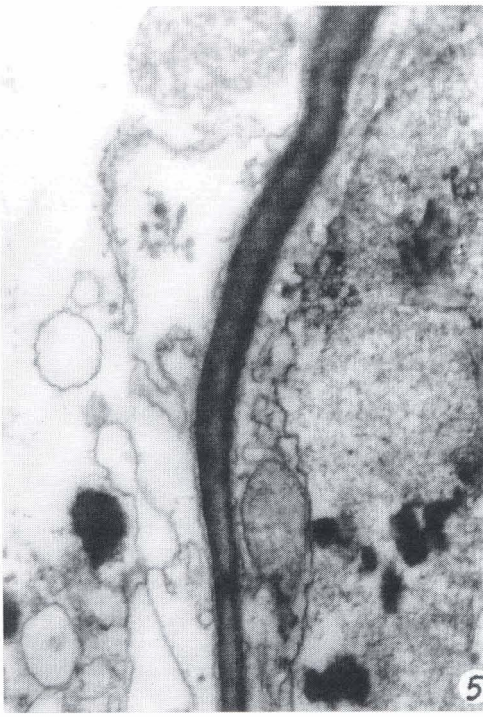


Plate I. Electron micrographs of the cells of tobacco regenerants obtained on MET and subcultivated on MET.
Fig. 1. (20000 ×), Fig. 2 (21000 ×), Fig. 3 (18100 ×).





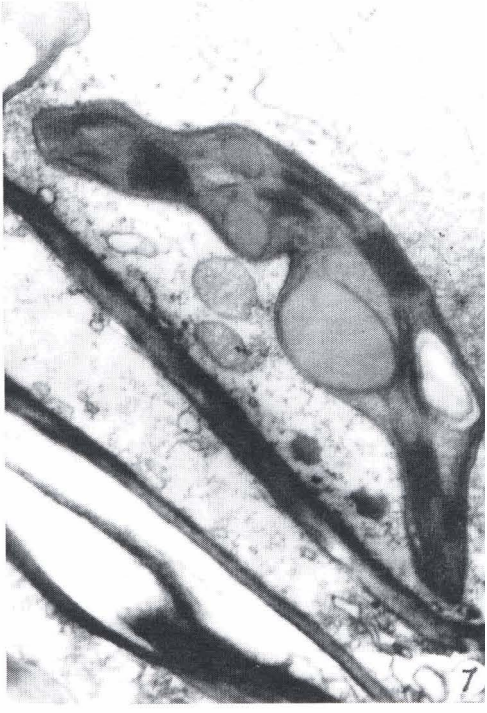


Plate II. Electron micrographs of the cells of tobacco regenerants obtained on MET and subcultivated on MET + 5% PEG.
Fig. 4. (10500 ×), Fig. 5. (30800 ×), Fig. 6. (9400 x), Fig. 7. (16100 ×).

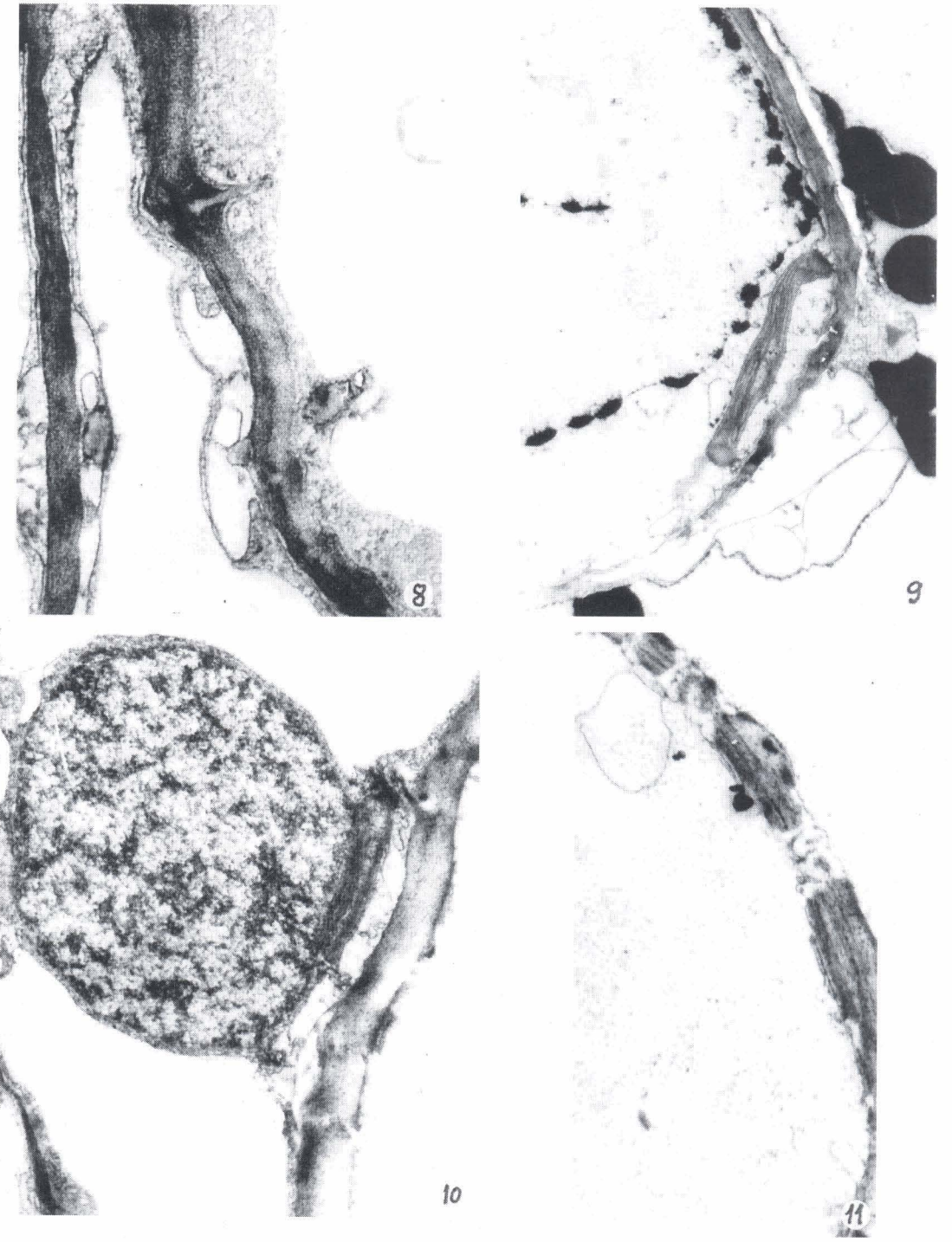
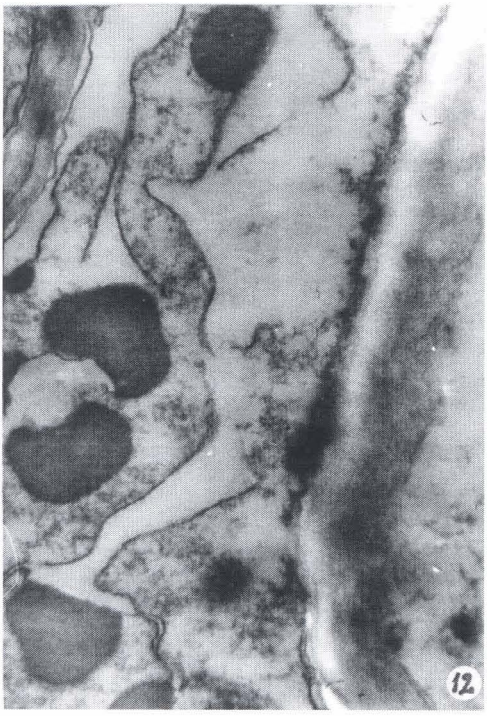
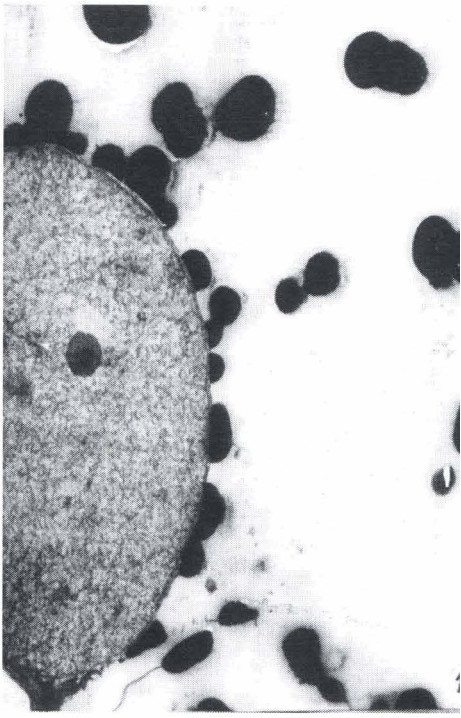
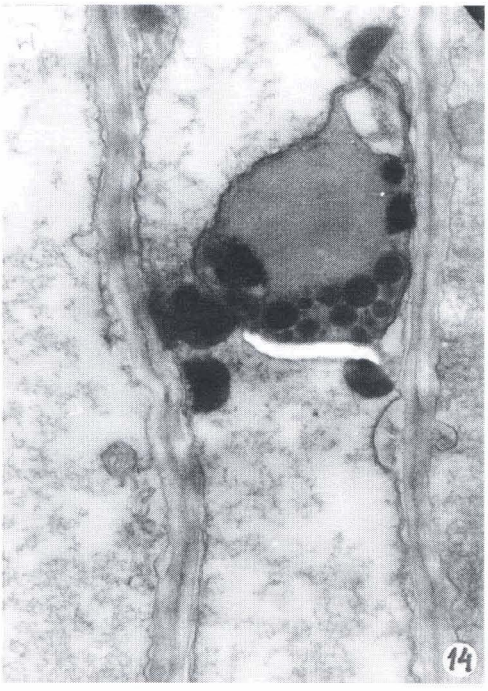


Plate III. Electron micrographs of the cells of tobacco regenerants obtained on MET and subcultivated on MET + 10% PEG.
Fig. 8. (15000 ×), Fig. 9. (18000 ×), Fig.10. (45000 ×, Fig.11. (20000 ×).







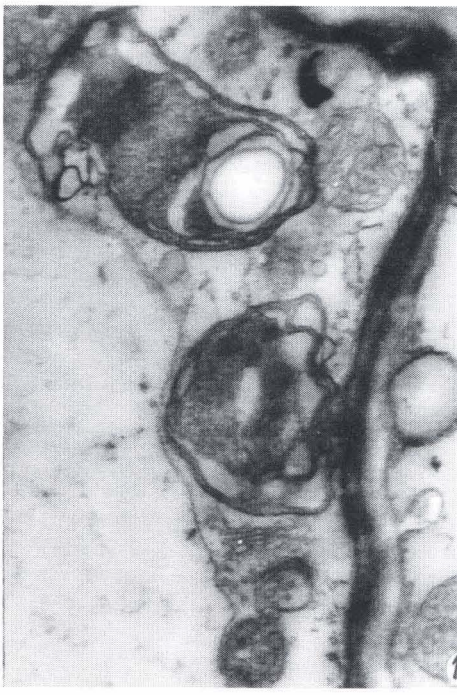


Plate IV. Electron micrographs of the cells of tobacco regenerants obtained on MET + 5% PEG and subcultivated on MET.
Fig.12. (29400 ×), Fig.13. (23800 ×), Fig.14. (30000 ×), Fig.15. (37000 ×).

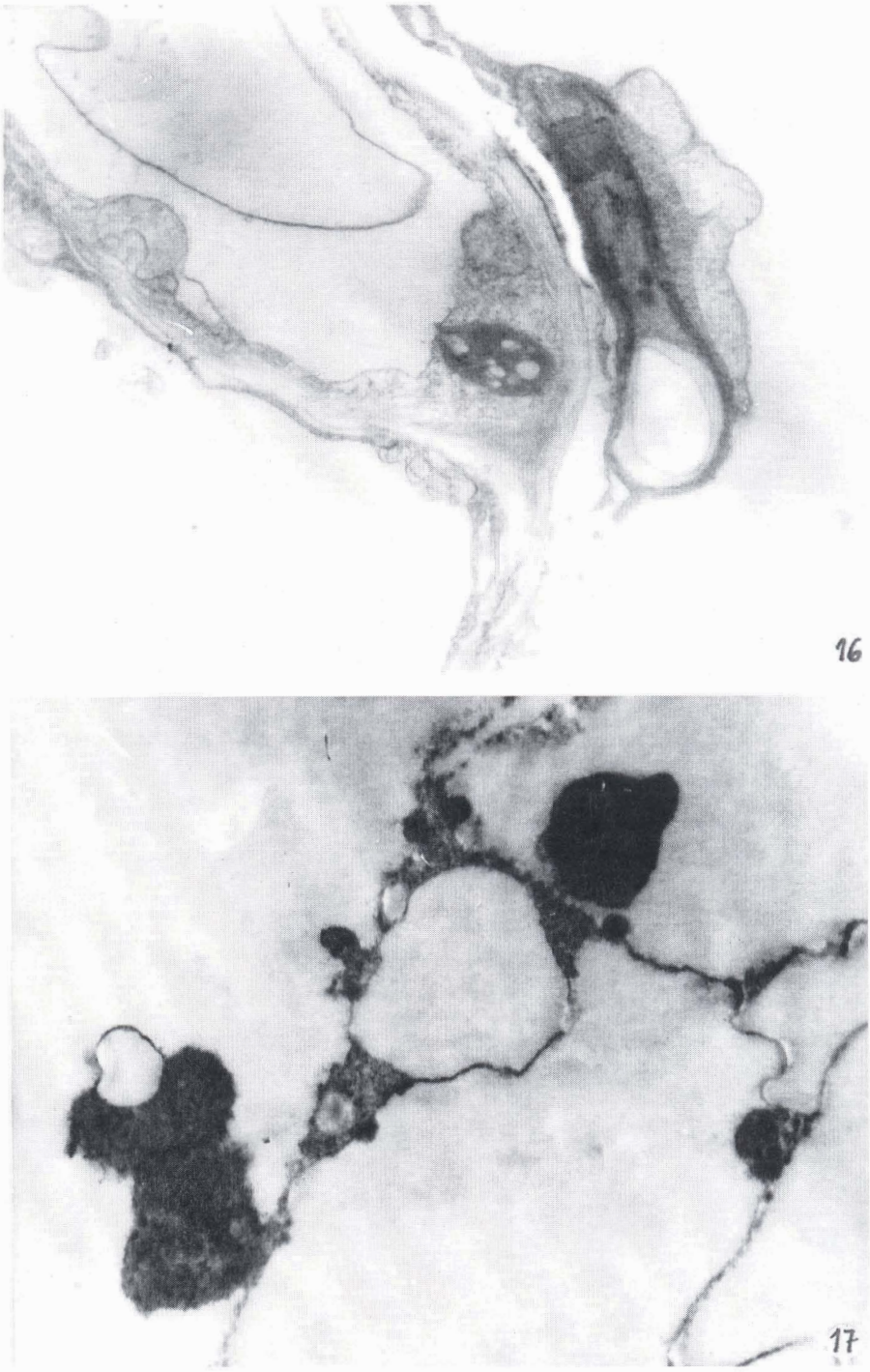
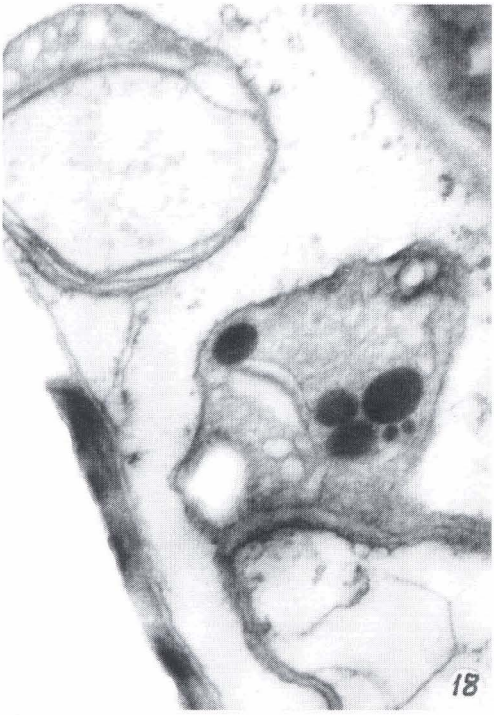
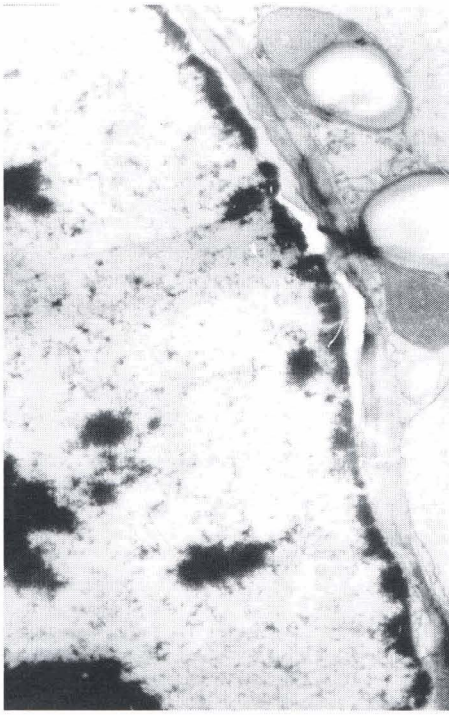
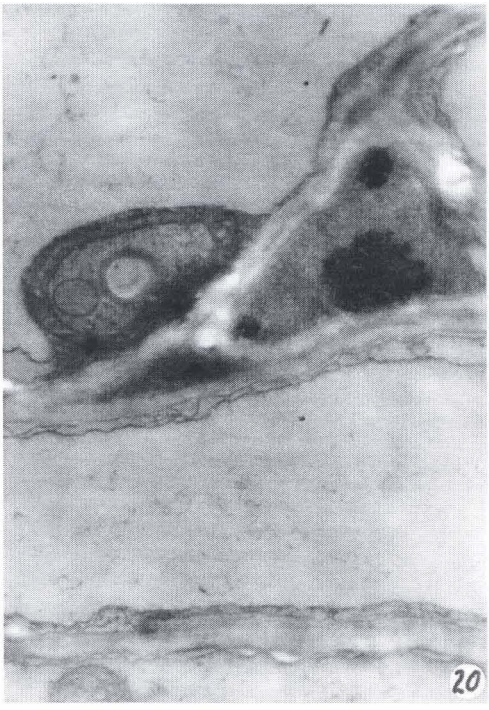


Plate V. Electron micrographs of the cells of tobacco regenerants obtained on MET + 5% PEG and subcultivated on MET + 5% PEG.
Fig.16. (26200 ×), Fig.17. (28000 ×).







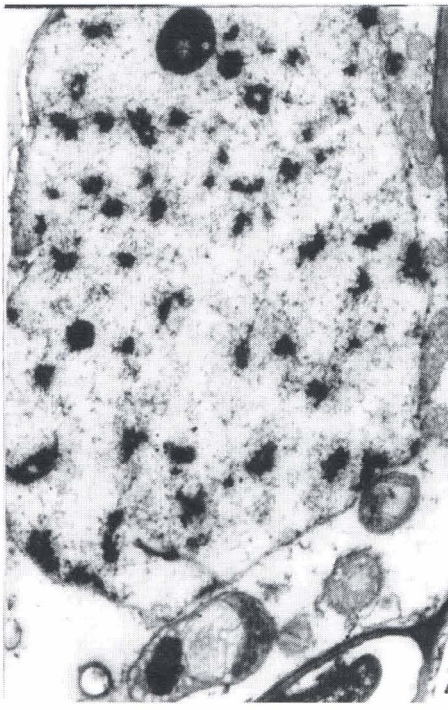


Plate VI. Electron micrographs of the cells of tobacco regenerants obtained on MET + 5% PEG and subcultivated on MET + 10 % PEG.
Fig.18. (15000 ×), Fig.19. (46000 ×), Fig.20. (24000 ×), Fig.21. (18000 ×).

– The cells of the tobacco regenerants obtained and subcultivated on the control culture medium (MET culture medium) presented in time certain ultrastructural modifications associated with senescence.

– The cells of the tobacco regenerants obtained on the control medium and subcultivated for 90 days on MET culture medium supplemented with PEG contained plastids with some thylakoid structure, aberrant mitochondria, as well as numerous secondary metabolites, which may be interpreted as an adaptive response to drought.

– The PEG successive stress caused additional effects, in direct correlation with the PEG concentrations: it was enhancing the organelle structure damages, the vacuolization extension and the presence of massive, opaque to electrons deposits.

– Associated with the drought stress the plastids with a disorganized structure contained a large number of plastoglobules, amyliferous inclusions, peripheral reticule, as well as, massive polyphenol accumulations.

– All modifications observed at the ultrastructural level confirmed the poor tolerance of tobacco cells to the successive PEG stress and explained the reduction of the morphogenesis potential of the tobacco stressed plants.

REFERENCES

1. Bohnert H.J., 1995, Adaptation to environmental stress. *The Plant Cell*, 7: 1099–1111.
2. Bohnert H.J. & Sheveleva E., 1998, Plant stress adaptations making metabolism move. *Current Opinion in Plant Biology*. **1**: 267–274.
3. Chartzoulaki K., Patakas A., Bosabalides AM., 1999, Changes in water relations photosynthesis and leaf anatomy induced by intermittent drought in two olive cultivars. *Environmental and Experimental Botany*, **42**: 113–120.
4. Chartzoulaki K., Patakas A., Kofidis G., Bosabalides AM., Nastou A., 2002, Water stress affects leaf anatomy gas exchange, water relations and growth of two avocado cultivars. *Scientia Horticulturae*, **95**:39–50.
5. Dumitrescu Rodica, 2003, Ultrastructural modifications induced by the hydric stress in the callus cells of Nicotiana tabacum L. (cv. Xanthi). Proceedings of the Institute of Biology, **V:** 471–483.
6. Dumitrescu Rodica, 2004, The response of petiole cells of Nicotiana tabacum L. (cv. Xanthi) to polyethyleneglycol 6000 and abscisic acid (hydric stress mediators). *Proceedings of the Institute of Biology*, **VI**: 381–391.
7. Galinski E., 1993, Compatible solutes of halophylic eubacteria . Molecular principles water solute interactions stress protection. *Experentia*, **49**: 487–486.
8. Maron R., Fahn A., 1979, Ultrastructure and development of oil cells in Laurus nobilis L. leaves. *Botanical Journal of the Linnean Society*, **78**: 31–40.
9. Melkonian J.J. *et al.* 1982, Determination of the volumetric modules of elasticity of wheat leaves by pressure-volume relations and the effect of drought conditions. *Crop. Sci.* **22**: 116–123.
10. Murashige T.& Skoog F., 1962, A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant*, **15**: 473–493.

11. Reynolds E.S., 1963, The use of lead cehats at high pH as an electronoopaque stain in electr microscopy. *J. Cell. Biol.*, **17:** 208 p.
12. Skriner K. & Mundi J., 1990, Gene expression in response to abscisic acid and osmotic stre *Plant Cell.*, **2**: 503–512.
13. Tyree M.T. & Jarvis P.G., 1982, Water in tissue and cells. In: *Encyclopedia of Plant Physiolo* **12**.B. Edited by O.L. Lange, P.S. Nobel, C.B. Osmond and H. Zeigler, Springer-Verl Berlin, 35–72.