STRESS PROTEINS AND ULTRASTRUCTURAL CHARACTERISTICS OF LEAF CELLS OF PLANTS WITH DIFFERENT TYPES OF ECOLOGICAL STRATEGIES

I. Kosakivska[1*], D. Klymchuk[1], V. Negretzky[1], D. Bluma[1], A. Ustinova[1]

[1]Institute of Botany of the National Academy of Sciences of Ukraine, 2 Tereschenkivska str., 01601, Kyiv-1, Ukraine

Summary. Stress proteins biosynthesis, DNA and RNA content, electron microscopy of leaf cells from plants with different types of ecological strategies have been studied. *Brassica campestris* var.olifera and *Amaranthus caudathus* L. were selected as ruderals. *Rumex patienta* L. x *Rumex tianshanicus* A.Los. was selected as stress-tolerant. Biosynthesis of stress proteins, DNA and RNA content, the ultra structural organization of leaf cells were analyzed after short-term heat (+40 ºC) and cold (+2 ºC) temperature stresses. The differences in protein spectra and nuclear acid content were revealed. Active synthesis of 50 kDa HSP and some low molecular weight proteins took place after temperature stresses. Stressful polypeptides were detected, one of them could be reviewed as a biomarker. Ultrastructural investigations revealed different features and directions in changes of cell organelles from plants with different types of ecological strategies under stress conditions. Perspectives of proteins usage as biomarkers of plants with different types of ecological strategies and probable role of ultrastructural changes in cell organelles during adaptation to environmental stresses are discussed.

Key words: biomarkers, ecological strategy, nucleic acids, stress

*Corresponding author, e-mail: science@botany.kiev.ua

proteins, ultra structure.
***Abbreviations*:** HSPs – heat shock proteins.

INTRODUCTION

The effect of environmental stresses on plants has been one of the main interests in modern biological research. This is connected with radical ecological changes and reduction in biodiversity. Changes driven by ecological stresses can be investigated at molecular and other hierarchical levels. Of particular interest is adaptation of protein biosynthesis and ultra structure to anomalous ecological factors. Such stresses as drought, salt, cold, heat, chemical pollutants and others, frequently act together and trigger adaptive and protective mechanisms (Vinocur, Altman, 2005). Stress proteins are critical to maintaining homeostasis under stress (Wang et al., 2004). The up regulation of stress proteins, which occurs against a background of depressive changes in polypeptide formation, relative to normal environmental conditions, is one of the main components of the adaptive response (Lorimer, 2001; Kosakivska, 2008). The genome (DNA) is stable through many stress, the transcriptome (mRNA population) and proteome (protein population) change during development, under biotic and abiotic stress, sometimes rapidly and dramatically (Watson et al., 2003; Rampitsek, Srinivasan, 2006). In our investigation we compared data obtained from DNA and RNA contents and proteins composition, examined changes in plant proteoms to further understanding of the role of proteins in realization of ecological strategy.

MATERIAL AND METHODS

To select potential biomarkers we screened plants with different types of ecological strategies. We focused on selecting species with agricultural or alternative energy source potential drawn both from the local flora and from among introduced plants. *Brassica campestris var. olifera* (stubble turnip) and *Amaranthus caudathus L.* (amaranth) were chosen from among ruderals.

B. campestris is annual light-requiring winter hardy culture that does not tolerate extended flooding and perished under ice. It utilizes C-3 type photosynthesis.

A. caudathus – an annual ecologically plastic culture with C-4 type photosynthesis. The geographic origin for the majority of species of *A. caudathus* is thought to be in North and "Tropical" America, whence this ancient grain culture migrated to Central America, Asia, Africa, Australia and Europe. This plant is undemanding to soil, possesses drought- salt- and disease resistance, and can easily adapt to various ecological conditions.

A new culture – hybrid *Rumex patienta L. x R. tianshanicus* A. Los was selected from among stress-tolerants. It is a perennial (up to 10 years) plant, characterized by high ecological plasticity, cold- and winter hardiness, and tolerance to salt and increased humidity.

Seeds of the selected species were first calibrated, and then 50 seeds of each species were transferred onto filter paper placed in Petri dishes kept in the dark at 25 ºC for germination. After two days, the seedlings were transferred to Hellrigel's nutrient solution under lighted conditions and grown for 4 days at 25 ºC and 2500 lx (14 h – light, 10 h – darkness).

To study biosynthesis under unfavourable conditions, 7-d-old seedlings were subjected to short term temperature stresses: 2 h – at 40 ºC and 2 h – at 4 ºC. Green parts of seedlings were used for protein extraction. Protein content was determined using the method of Bradford (Bradford, 1976).

Proteins were separated in 13 % SDS-PAGE following Leammli procedure (Laemmli, 1970). Standard proteins with molecular weights of 200, 150, 120, 100, 85, 70, 60, 50, 40, 30, 25, 15, 10 kD were used as markers. We used the Total Lab 2.1 package to track and tabulate the distribution of molecular weights.

We analysed nucleic acid content in all studied plants. Nucleic acids were extracted using the reagent Trizol ("Sigma", USA) following manufactures recommendations. The concentration of RNA and DNA was determined using a "Nanodrop" UV- spectrophotometer at 260 and 280 nm. Quality, integrity and concentration of RNA samples were analyzed using an "Agilent 2100" bioanalizer and integrating chips "NanoChip" according to manufactures recommendations. Isolated RNA was characterized using electrophoresis on biochips with an "Agilent 2100" bioanalyzer (Negretzky

et al., 2007).

For electron microscopy analysis seedling samples were fixed in a solution of 2.5 % (v/v) glutaraldehyde in 0.05 M sodium cacodylate buffer, pH 7.2 for 3 h, followed by 1 h-incubation in 1 % (v/v) osmium tetroxide in the same buffer. The specimens were dehydrated in a graded ethanol series and embedded in Epon 812-Araldite resins. Silver-gold sections (60±10 nm) were obtained with LKB 8800 ultramicrotome, collected on formvar-coated copper grids, stained with uranyl-lead citrate (Reynolds, 1963). Specimens were then examined in a JEM-I230 transmission electron microscope at 80 kV (JEOL, Japan).

RESULTS AND DISCUSSION

100 % of *B. campestris* and *A. caudathus* seeds germinated.

100 % of *Rumex patienta L. x R. tianshanicus* seeds also germinated, but further seedling growth was hindered by incomplete seed coat separation.

Recall that *B. campestris* and *A. caudathus* followed the ruderal strategy. Ruderals grow in reduced competition environment, are characterized by rapid growth in favourable conditions and switch to seed production under stresses. Ruderals stop vegetative growth, shorten or eliminate juvenile phases, and hence accelerate flowering and seed formation. Under favourable conditions and in the absence of a competition they are characterized by fast growth, particularly of root system (Grime, 1974).

PROTEIN CONTENT UNDER TEMPERATURE STRESS

We determined that under stress conditions the ruderals contained three times the total proteins compared to the stress-tolerants. In *A. caudathus* a ruderal with C-4 type photosynthesis proteins content under a short-term cold stress was unchanged. A short-term heat shock caused an insignificant reduction of proteins content. In *B. campestris* – a ruderal with C-3 type photosynthesis substantially increased protein synthesis under short-term cold stress, while a short-term heat shock lead to reduced proteins content This showed that plants with different routes of carbon incorporation can respond to stress differently even when they use the same ecological

strategies. Protein biosynthesis in plants with C-4 type of photosynthesis appeared to be more stable (Fig. 1).

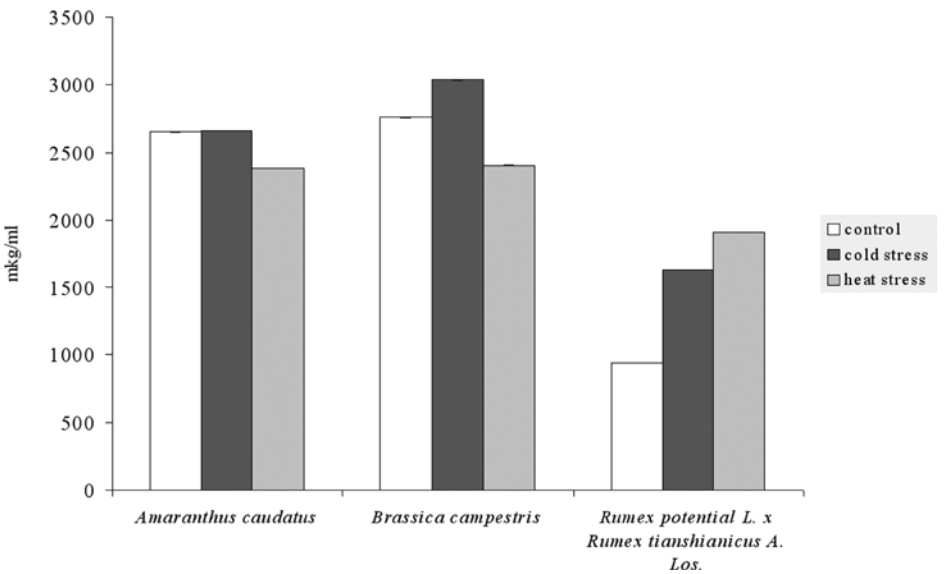


Fig. 1. Protein content under temperature stress.

Interesting results were obtained for *Rumex patienta L. x R. tianshanicus,* a stress-tolerant. Stress-tolerants stop vegetative growth under stress, and slow down the transition to flowering, focusing the resources available to the plant on the processes of adaptation (Grime, 1974). Under the conditions of short-term heat stress we observed a 2-fold increase of protein content in seedlings. A short-term cold stress also caused substantial increase in protein content in these plants (Fig. 1). Thus, protein synthesis in stress-tolerants appears to be considerably more sensitive to temperature stresses, than in ruderals. This may be due to the activation of a stress response mechanism which in turns upregulates protein synthesis.

BIOSYNTHESIS OF STRESS PROTEINS

Using polyacrylamide gel electrophoresis (13 %) we investigated the protein spectra of the above-ground parts of *B. campestris*, *A. caudathus*

and *Rumex patienta L. x R. tianshanicus* under control and stress conditions (Fig. 2).

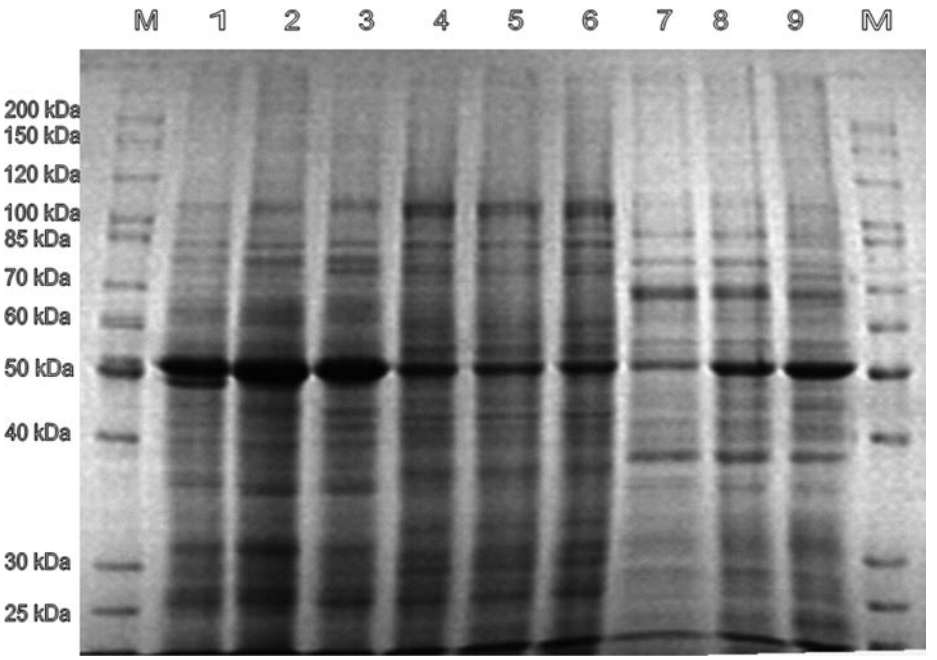


Fig. 2. Protein spectra of the above-ground parts of *B. campestris* (1-3)*, A. caudathus* (4-6) and *Rumex patienta L. x R. tianshanicus* (7-9) under control and stress conditions (1, 4, 7 – control; 2, 5, 8 – cold stress, 2 h, 4 ºC; 3, 6, 9 – heat stress, 2 h, 40 ºC).

Protein spectra differed under both control and stress conditions. Ruderals were characterized by the presence of 50 kDa polypeptide in control conditions, with elevated production under stress in *B. campestris*, whereas the level of such polypeptide in *A. caudathus* remained effectively unchanged between control and stress conditions. In *Rumex patienta L. x R. tianshanicus* seedlings the biosynthesis of 50 kDa polypeptide was considerably amplified during heat shock. The reaction to short-term cold stress was also quite strong, but less pronounced.

Changes due to temperature stresses took place across the entire spectrum of molecular weights. *B. campestris* seedlings under temperature stresses

synthesized 72 kDa polypeptides; *A. caudathus* seedlings synthesized 94 kDa polypeptides.

The effect of temperature stress on protein synthesis in *Rumex patienta L. x R. tianshanicus* seedlings was more dramatic. Indeed, temperature stresses caused the synthesis of a *de novo* 71 kDa polypeptide and an increase in 44, 78 and 109 kDa proteins.

In *B. campestris* seedlings the reduction in 47 kDa protein content was observed under temperature stresses. Heat stress was assosiated with ramped-up synthesis of low molecular weight 22-25 kDa HSP in *Rumex patienta L. x R. tianshanicus* seedlings. Under short-term cold stress the content of 32 kDa polypeptide increased in *B. campestris* seedlings. A less pronounced increase was also observed under heat shock. Temperature stress resulted in decreased production of 30-35 kDa HSP in *A. caudathus* seedlings, heat shock having a greater effect than the cold (Fig. 2).

We detected measurable differences in spectra of low molecular weight proteins between control and stress-induced conditions in ruderals. In *A. caudathus* seedlings, the plants with C-4 type photosynthesis, we observed a reduction of low molecular weight HSP synthesis, especially under a heat shock. At the same time the synthesis of low molecular weight HSP in *B. campestris* – the plant with C-3 type photosynthesis increased under heat shock (Fig. 2).

Several classes of HSPs have been described in plants, among them HSP 110, HSP90, HSP70, HSP60 and low molecular weight HSPs (Vierling, 1991).The main function of HSPs is to alter the conformation or assembly of other protein structures.Molecular chaperones are key components contributing to cellular homeostasis in cells under stress conditions. They are responsible for the protein folding, assembly, translocation and degradation (Knight and Ackerly, 2001). HSP 70 kDa family has essential functions in preventing aggrigation and assisting refolding of non-active proteins under both normal and stress conditions (Hartl, 1996; Frydman, 2001). It is well known, that members of HSP70 kDa family are mainly localized in cytoplasm, where both stress – induced and constitutive polypeptides are expressed (Bukau and Horwich, 1998; Morimoto, 1998). Members of this protein family can also be found in the nucleus and mitochondria. As our experiments show, the involving of HSP70 kDa family proteins in

temperature stress response is most pronounced in stress-tolerant, whose ecological adaptation strategy is directed towards a rapid realization of life cycle.

Stress proteins from the HSP 60 kDa family can be localized in mitochondria and chloroplasts both in stress-induced and normal conditions (Wang et al., 2004). The basic function of proteins from this family is to enable and assist conformational changes in mitochondrial polypeptides (Hamilton, Coleman, 2001). In ruderal species *B. campestris* and *A. caudathus*, HSP 60 kDa family proteins were present in significant amounts both under stress and in control conditions, whereas in the stress-tolerant *Rumex patienta L. x R. tianshanicus* only small amounts of these proteins were present in control. Temperature stresses significantly increased the content of HSP 60 kDa family proteins in the stress-tolerant species, which attests to a stress-induced transformation of the mitochondrial protein synthesis system under unfavourable conditions.

Revealed differences in proteins synthesis patterns in response to temperature stress among plants with different types of ecological strategy point to differences at molecular level. Gene activation and expression depend on the ecological strategy (Pierce et al., 2005). The synthesis of stress proteins in stress-tolerant plants occurs more intensive. Under temperature stresses the amount of HSPs in mitochondria and chloroplast increased. Fast adaptive reactions in stress-tolerant plants promote their survival in unfavourable conditions. The observed distinctions in protein synthesis patterns suggest that stress response proteins could be useful as biomarkers of different ecological strategies.

DNA AND RNA ANALYSES

We determined (Fig. 3) that heat shock caused a significant reduction in DNA content in *A. caudathus* seedlings, whereas the amount of DNA in *B. campestris* and *Rumex patienta L. x R. tianshanicus* seedlings remained essentially at the same level as control. After the application of cold stress the contents of DNA in *B. campestris* and *Rumex patienta L. x R. tianshanicus* seedlings decreased considerable. The level of DNA in *A. caudathus* seedlings practically did not change.

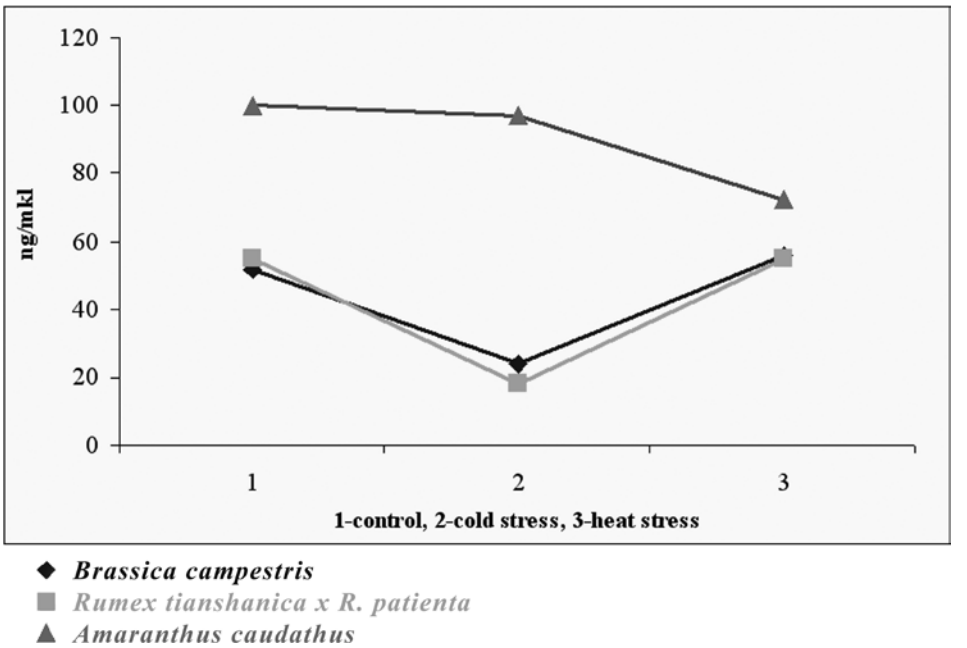


Fig. 3. DNA content in 7-day-old seedlings of *Brassica campestris, Rumex tianshanica x R. patienta, Amaranthus caudathus* under temperature stress (cold stress - 4 ºC, 2 h, heat stress - 40 ºC, 2 h).

RNA content (Fig. 4) significantly decreased under heat shock in *A. caudathus* and *B. campestris* seedlings, whereas at *Rumex patienta L. x R. tianshanicus* did not change. Cold stress caused a significant reduction of RNA level in *B. campestris* seedlings. The level of RNA in *Rumex patienta L. x R. tianshanicus* remained stable.

We determined that nucleic acid content varied between plants from using the same adaptive strategy and those using different strategies. In particular, in seedlings of stress-tolerant *Rumex patienta L. x R. tianshanicus* the amount of RNA under temperature stresses was practically unchanged from the control conditions.

In a C-4 type photosynthesis ruderal – *A. caudathus* as in C-3 type photosynthesis *B. campestris*, the contents of RNA considerably decreased under heat stresses. RNA level was relatively more stable under cold stress in *A. caudathus*. The content of nucleic acids in plant with C-4 – type

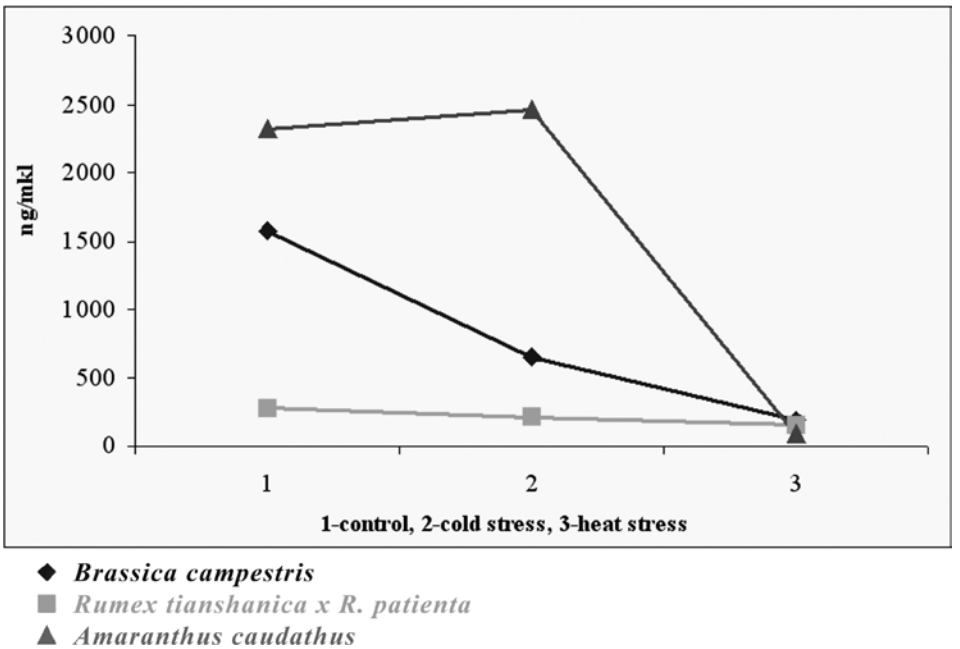


Fig. 4. RNA content in *Brassica campestris, Rumex tianshanica x R. patienta, Amaranthus caudathus* under cold and heat stress (cold stress – 4 ºC, 2 h, heat stress – 40 ºC, 2 h).

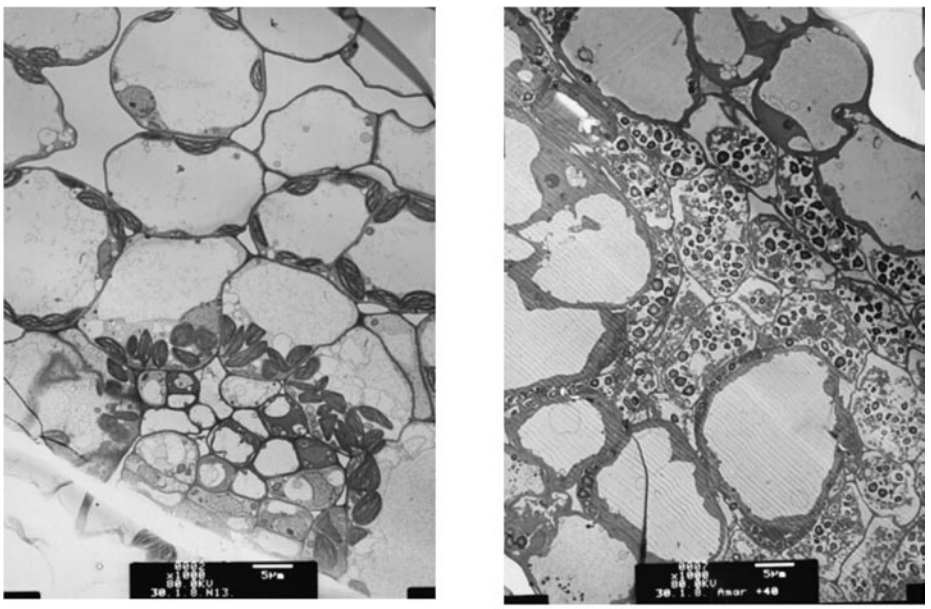
photosynthesis rapidly decreased under heat stress.

The retention of near-control RNA level under temperature stresses reflects upon the stability of biosynthetic processes in a given plants in unfavourable conditions.

ELECTRON MICROSCOPY ANALYSES

Together with study of stress proteins biosynthesis we performed an electron microscopy analysis of 7-day-old *B. campestris* and *A. caudathus* seedlings exposed to short-term temperature stresses (Fig. 5-6). We determined that starch content in *B. campestris* cells had dropped significantly under heat shock, whereas the opposite was true in *A. caudathus* cells. This phenomenon may be connected with the reduction of carbohydrate outflow from cells of plants with C-4 type photosynthesis

under heat stress. It was shown, that mesophyll and bundle sheath cell areas decreased under water stress. Electron microscopy studies revealed that in bundle sheath cell chloroplasts under stress conditions the amount of starch increased, changes in thylakoids orientation also took place and a reduction in chloroplast area was observed. Stressfull mesophyll chloroplasts were characterized by increasing of peripheral reticulum and starch granules and decreasing of grana stacking amount related to a decreasing of leaf sodium concentration. The number of mitochondria per mesophyll cell increased under water stress (Utrillas, Alegre, 1997).



Control Heat stress

Fig. 5-6. Electron microscopy of mesophyll cells from 7-day-old *Amaranthus caudathus* seedlings.

Thus, we revealed an ultra structural difference in how plants that have the same ecological strategy, but different types of photosynthesis respond to temperature stresses.

CONCLUSIONS:

Proteins, including stress proteins, can be used as biomarkers for studying plants with different types of ecological strategy.

– The patterns of gene expression and activation of biosynthesis of stress proteins depend on the type of ecological strategy;

– The results of our ultrastructural analysis suggest that such analyses hold promise for the study of structural adaptive changes in plants with different ecological strategies;

– Analyses of biosynthetic, structural and functional patterns can help further our understanding of how different ecological strategies are implemented in plants and what roles they play in the preservation and restoration of the biodiversity in the era of global-scale ecological changes.

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