Protoplasma (1988) 144: 1–9

PROTOPLASMA

Observations of Soybean Root Meristematic Cells in Response to Heat Shock

YUNG-REUI CHEN, MEI CHOU, SHAU-SHI REN, YIH-MING CHEN, and CHU-YUNG LIN*

Department of Botany, National Taiwan University, Taipei, Taiwan

Received November 5, 1986
Accepted January 25, 1988

Summary

Soybean seedlings (*Glycine max*) were incubated in narrow temperature regimes to study the effects of heat shock on cell structures. The incubation temperatures used were as follows: 1. 28 °C (2 h); 2. 40 °C (2 h); 3. 45 °C (2 h); 4. 40 °C (2 h) → 45 °C (2 h); 5. 47.5 °C (10 min); 6. 40 °C (2 h) → 47.5 °C (10 min). Both optical and electron micrographs were taken of the different tissues of root meristems as they responded to heat shock. Cells of roots heated to 45 °C (2 h) or 47.5 °C (10 min) with lethal treatment showed drastic heat injuries: *e.g.*, membrane damage, coagulated plasmolysis, protoplasmic contraction, and leakage of cell content. Nucleolar segregation occurred in cells treated at both lethal and supraoptimal temperatures. Seedlings preincubated at 40 °C (2 h) became thermo-tolerant to lethal temperature treatment of 45 °C (2 h) or 47.5 °C (10 min), by protecting the plasmalemma, mitochondria, plastids and nuclei from heat damage. Without preincubation, however, these structures were destroyed.

Keywords: Heat shock; Soybean root; Ultrastructure.

Abbreviations: CC Central cylinder; CR Cortex; M Mitochondria; N Nuclei; Nu Nucleoli; P Plastids; RC Root cap; RE Region of elongation; RM Region of meristem.

1. Introduction

High temperature injures plant cells, and the extent of injury is releated to the duration of heat treatment (reviewed by LEVITT 1980). Observations with a light microscope revealed cell injury caused by exposure to high temperature including irreversible plasmolysis, organelle coagulation, protoplasmic constriction, and disorganization of membrane systems (DANGEARD 1951, ALEXANDROV 1964, DANIELL *et al.* 1969). Observations with an electron microscope revealed: decrease of nuclear volume (BARABAL'CHUK and CHERNYAVSKAYA 1975), chromatin degeneration (DAS 1973), nucleolar segregation (RISUEÑO *et al.* 1973), membrane breakage (SKOGGVIST 1974), membrane curl in the mitochondria (SKOGGVIST 1974), structural alteration of the thylakoid membrane (KRAUSE and SANTORIUS 1975) and the loss of 70 S ribosomes in the plastids (SCHAEFERS and FEIERABEND 1976).

The repair of plant cells exposed to reversible heat damage, after having been returned to an optimal growth temperature, has been reported (BAUER and SENGER 1979). The mechanism of heat damage repair is still unknown, but the degree of recovery and the time required for recovery depend upon the severity of the heat stress (BERRY and BJÖRKMAN 1980).

Soybean seedlings briefly exposed to a lethal temperature followed with an incubation at normal growth temperature, or exposed to optimal heat shock temperatures for several hours became thermotolerant to otherwise lethal temperature treatments (LIN *et al.* 1984).

This study investigated the ultrastructural changes caused by various heat shock treatments (thermoprotective versus non-thermoprotective) applied to soybean seedlings for understanding the acquisition of thermotolerance at cellular levels.

2. Materials and Methods

2.1. Germination and Heat Treatment

Soybean seeds (*Glycine max* cv Taita Kaoshung no. 3) were germinated in rolls of moist tissue paper at 28 °C in the dark. Three-day-

* Correspondence and Reprints: Department of Botany, National Taiwan University, Taipei, Taiwan, R.O.C.

old seedlings were treated in a shaking water bath as follows: 1. 28 °C (2 h); 2. 40 °C (2 h);3. 45 °C (2 h); 4. 40 °C (2 h) → 45 °C (2 h); 5. 47.5 °C (10 min); 6. 40 °C (2 h) → 47.5 °C (10 min).

2.2. Electron Microscopy

Segments approximately 1.2 mm from the tips of the seedling roots were fixed in 3% glutaraldehyde (0.1 M cacodylate buffer, pH 7.2) for 3 h at room temperature. They were then washed three times in buffers changed every 30 min. The washed samples were postfixed in 1% osmium tetraoxide for 4 h and were kept in ice water. They were then rewashed three times with distilled water of 30 min each. Finally, the samples were dehydrated in a graded series of ethanol, and were infiltrated with different gradients of absolute ethanol and Spurr's resin (1969, 3:1, 1:1, 1:3, respectively) for 1 h. The samples were placed in pure resin twice (1 h each) and were finally placed in fresh resin overnight. Then transferred to a fresh medium of pure resin and were polymerized at 70 °C for 8 h. Thin sections were cut on an LKB Ultratome V and a Sorvar MT 5000 with glass and diamond knives. After being stained with saturated methanolic uranyl acetate for 20 min and lead citrate for 5 min, sections were examined, and photographed under a Hitachi H-600 transmission electron microsope at an acceleration voltage of 75 kV.

For observation with a light microscope, 1 μm semithin sections were stained with 1% toludine blue, mounted with mineral oil, and observed under the microscope. Photographs were taken with a Zeiss photomicroscope III using Kodak Panatomic-X film.

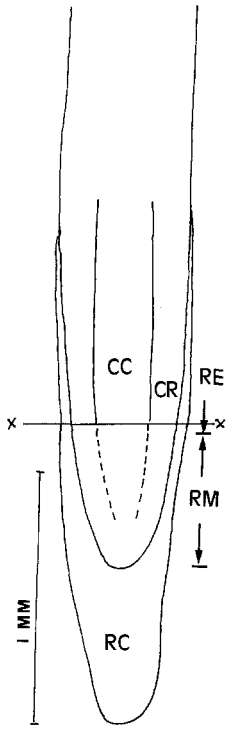

Fig. 1. Diagram of soybean root and the approximate planes of sectioning (× − − − ×)

3. Results

3.1. 28 °C (2 h)

Fig. 1 shows the diagram of soybean root and the approximate planes of sectioning obtained for this study. As shown in Fig. 2*A* the root cap from the tip of the root had 2–4 layers of cells and always showed plasmolysis. The protoderm, enclosed by the root tip, was a layer of hexagonal cells compactly arranged; it had a clear lineage distinctly different from outer root cap. The ground meristem consisted of a group of parenchyma cells, spherical to oblong in shape and variable in size. Intercellular spaces occurred between the cells of the ground meristem. Procambial cells were much more compact and were much smaller than those of the ground meristem; their shapes were diversified (Fig. 2*B*). The staining of the cells in the potential phloem region was more dense, and there were sometimes 2 nuclei within one cell. Many miniature vacuoles, or small vacuoles containing residuals of protein bodies, were found in cells of the protoderm, ground meristem, and procambium (Figs. 2*A* and *B*). Plastids containing starch were also frequently observed in the cells of various tissues. All meristematic cells had prominent nuclei with a distinct nucleolus, and the ratio of the volume of the nucleus to the volume of the cell was greatest in the procambium. This ratio decreased from the out-ground meristem to the protoderm and was lowest in the inner and middle ground meristem. Cell divisions were found in cells of the meristem, and were more often found in the procambium.

The meristematic cells had an electron-transparent cell wall surrounding the outside of the protoplasts. The plasmodesmata between two cells often penetrated the cell wall (Fig. 3*A*). The plasmalemma were mostly smooth and sometimes had an inward curvature (Fig. 3*A*, arrow), indicating the occurrence of endocytosis and exocytosis in the meristematic cells. Narrow cisternae with flatted sacs or branched tubules were randomly distributed in the endoplasmic reticulum (Fig. 4*A*, arrowhead) (ER). Both ameboid plastids and amyloplasts occurred in the meristematic cells (Fig. 4*A*). Mitochondria were evenly distributed in the cytoplasm. Small vacuoles were located in the peripheral or subcentral regions of the cytoplasm, and vacuolar fusions resulted in the formation of large vacuoles. Vacuolar inclusions, mainly of proteins bodies, frequently appeared in the meristematic cells of the root (Fig. 3*A*, arrowhead). The central nucleus had condensed chromatin in the region near the nuclear envelope, and had a prominent and compact nucleolus.

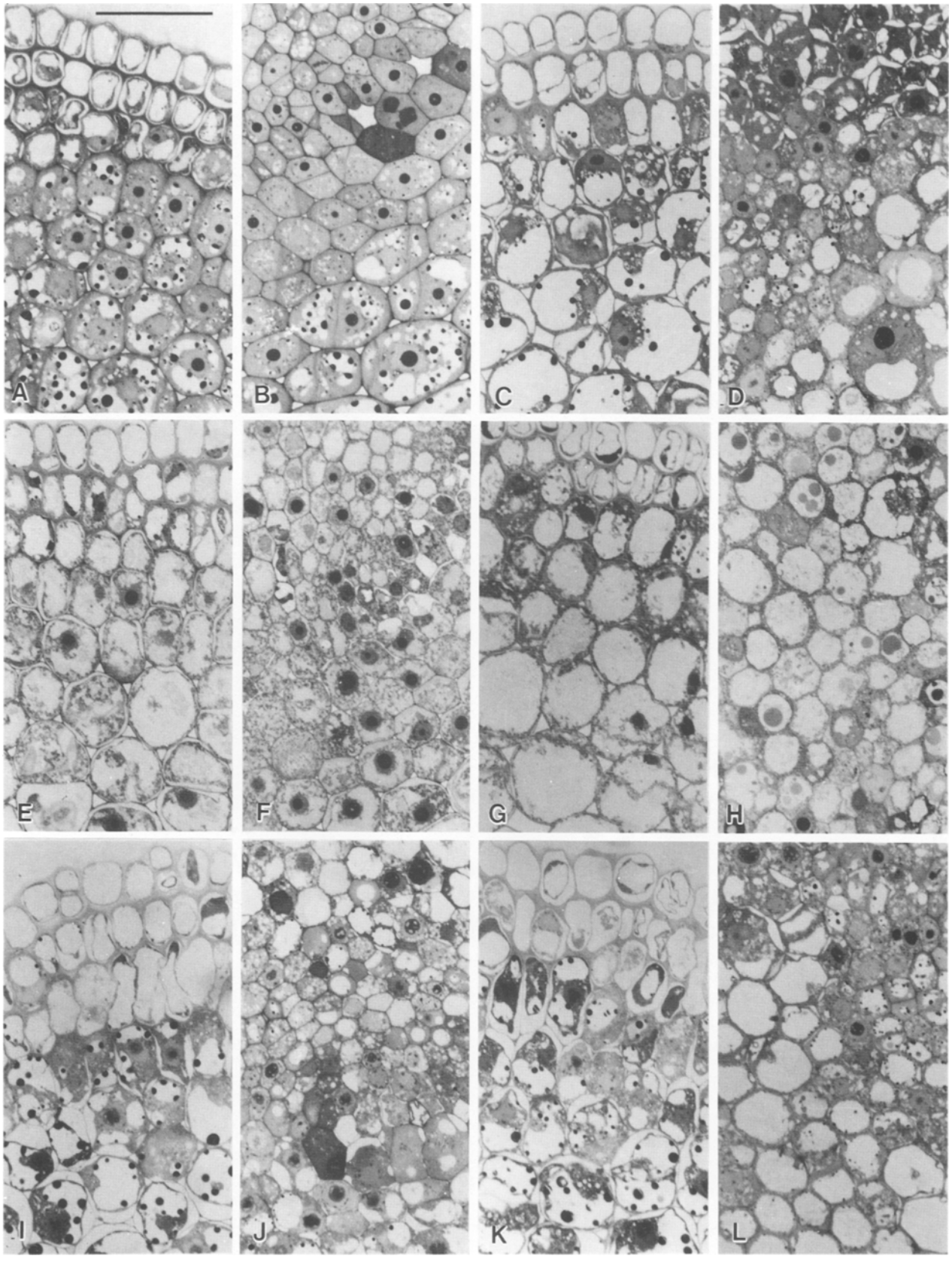

Fig. 2. Different tissues of meristematic cells in soybean roots responsed to different temperature treatments. *A*. Protoderm and peripheral ground tissue at 28 °C (2 h). *B*. Procambium and central ground tissue at 28 °C (2 h). *C*. Protoderm and peripheral ground tissue at 40 °C (2 h). *D*. Procambium and central ground tissue at 40 °C (2 h). *E*. Protoderm and peripheral ground tissue at 45 °C (2 h). *F*. Procambium and central ground tissue at 45 °C (2 h). *G*. Protoderm and peripheral ground tissue at 47.5 °C (10 min). *H*. Procambium and central ground tissue at 47.5 °C (10 min). *I*. Protoderm and peripheral ground tissue at 40 °C (2 h) and then 45 °C (2 h). *J*. Procambium and central ground tissue at 40 °C (2 h) and then 45 °C (2 h). *K*. Protoderm and peripheral ground tissue at 40 °C (2 h) and then 47.5 °C (10 min). *L*. Procambium and

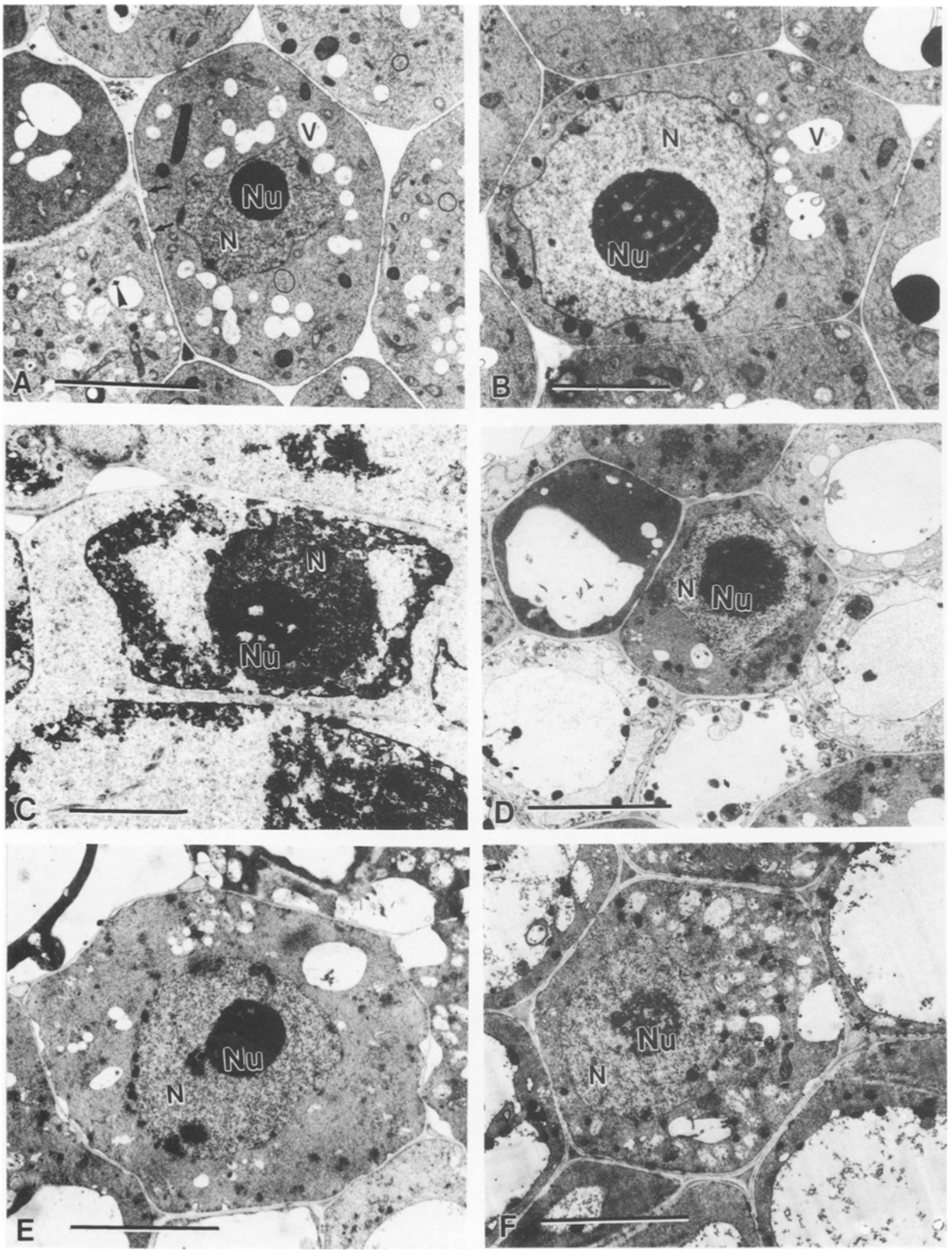

Fig. 3. Electronmicrographs of cell structure in meristematic cells of soybean root in response to different heat shock temperatures. *A*. 28 °C (2 h) – control. *B*. 40 °C (2 h). *C*. 45 °C (2 h). *D*. 47.5 °C (10 min). *E*. 40 °C (2 h) → 45 °C (2 h). *F*. 40 °C (2 h) → 47.5 °C (10 min)

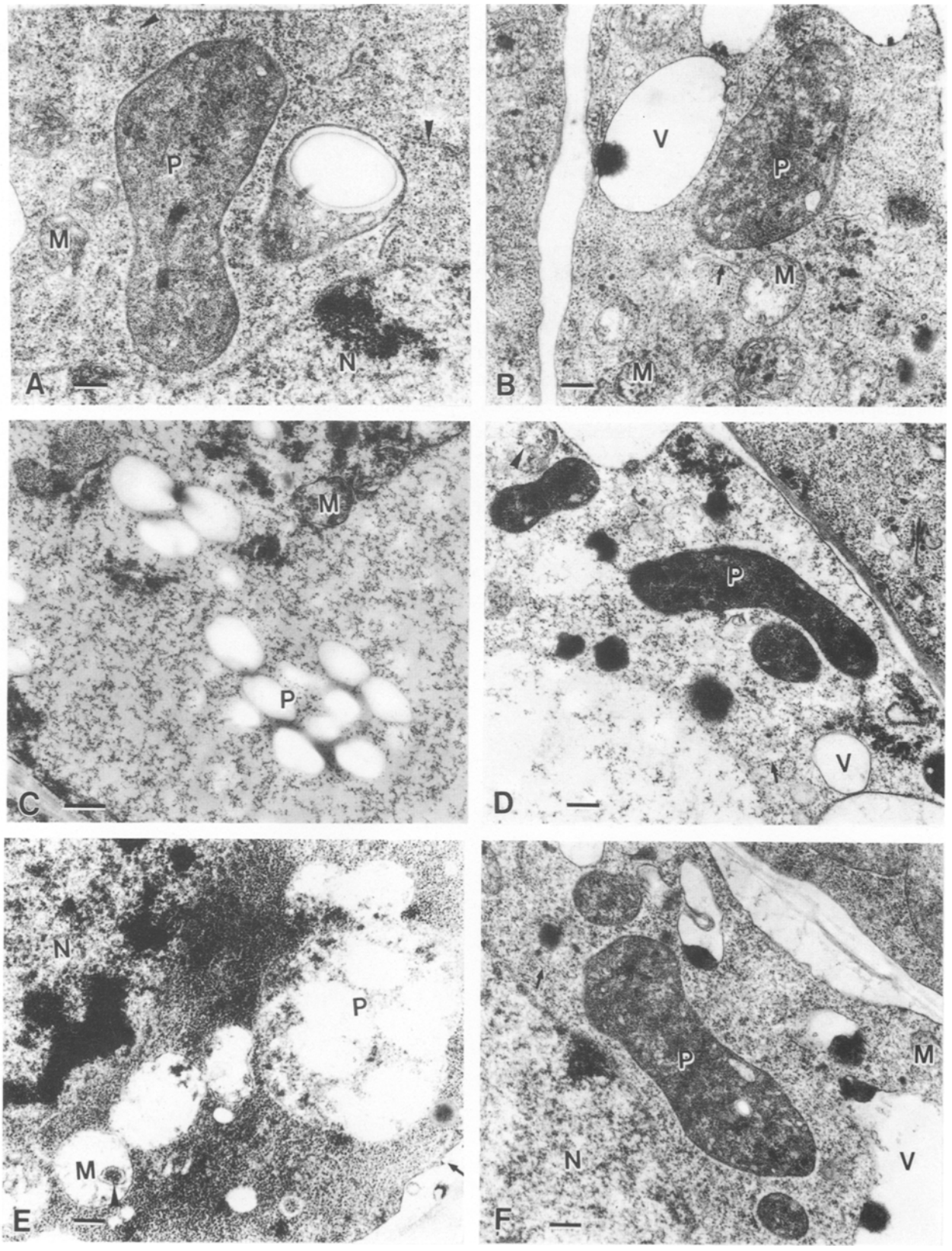

Fig. 4. Electronmicrographs of organelles in meristematic cells of soybean root in response to different heat shock temperatures. *A*. 28 °C (2 h) – control. *B*. 40 °C (2 h). *C*. 45 °C (2 h). *D*. 47.5 °C (10 min). *E*. 40 °C (2 h) → 45 °C (2 h). *F*. 40 °C (2 h) → 47.5 °C (10 min)

3.2. 40 °C (2 h)

The response of meristematic cells to optimal heat shock temperature (40 °C) was quite different in various tissues (Figs. 2*C* and *D*). Higher temperatures induced plasmolysis in all of the cells in the protoderm and ground meristem. In the procambium, only the region of potential vascular tissues was easily plasmolyzed. The stainability of the cytoplasm increased, and this may be related to cytoplasmic aggregation. The percentage of plasmolyzed cells in the central procambium decreased by 15–20% after treatment at 40 °C for 2 hours.

As shown in Fig. 3*B*, the plasmalemma of the central procambial cells were intact and were in close contact with the cell wall. There were no distinct changes in the morphology of the plastids, tonoplasts or vacuoles. However, there were several morphological changes noted (Fig. 4*B*). First, the cisternae of the endoplasmic reticulum expanded (Fig. 4*B*, arrow). Second, most mitochondria had well-organized crests, but the crests of some mitochondria were irregular, indicating that the mitochondrial population in the cytoplasm did not respond to heat shock in a uniform manner (Fig. 4*B*). Third, the nucleoplasm was more electron-transparent, and the nucleolus was more diffusely packed (Fig. 3*B*).

3.3. 45 °C (2 h)

After this lethal heat treatment, the roots were light in color and soft in texture. As shown in Figs. 2*E* and *F*, and Table 1, all cells in the root tip showed plasmolysis, cytoplasmic coagulation, vacuolar breakage, and an increase in the size of the nucleolus. The leakage of cell content might be related to membrane disruption or to changes in membrane permeability, which results in cell death. The deformities and irregularities noted in the fine structure were: membrane breakage in the plasmalemma, the endoplasmic reticulum, the mitochondria, the plastid and the nuclear envelope; nucleolar vacuolation and fragmentation; an increase in nucleus stainability and the leakage of cell substances (Figs. 3*C* and 4*C* and Table 2).

Table 1. *Different tissues of soybean root tip responsed to different heat treatment**

Meristems	Treatment	Plasmolysis	Cytoplasmic coagulation	Starch grains	Oil drops	Vacuolar intactness	Stainability of nucleus	Changes in nucleolar volume	Nucleolar vacuolation
Protoderm	28 °C (2 h)	−	−	+	−	+	+	−	−
	40 °C (2 h)	+, −	+, −	++	−	+, −	+++	+	+
	45 °C (2 h)	+++	+++	+++	−	−	+++	−	+
	47.5 °C (10 min)	+++	++	+++	−	−	+++	+	+
	40 °C → 45 °C (2 h) (2 h)	++	+, −	+++	−	+, −	+++	+	++
	40 °C → 47.5 °C (2 h) (10 min)	++	+, −	+++	−	+, −	+++	+	++
Ground meristem	28 °C (2 h)	−	−	++	−	+	+	−	+, −
	40 °C (2 h)	+, −	+, −	+++	−	+, −	++	+	+, −
	45 °C (2 h)	+++	+++	++	−	−	+++	+	+, −
	47.5 °C (10 min)	+++	+++	+++	+	−	++	+	+, −
	40 °C → 45 °C (2 h) (2 h)	++	+, −	+++	−	+, −	+	+	+, −
	40 °C → 47.5 °C (2 h) (10 min)	+, −	+, −	+++	−	+, −	+	+	+, −
Procambium	28 °C (2 h)	−	−	−	−	+	+	−	+, −
	40 °C (2 h)	+, −	+, −	+++	−	+	++	+	++
	45 °C (2 h)	+++	++	+	−	−	++	+	++
	47.5 °C (10 min)	+++	+++	+++	+	−	++	+	++
	40 °C → 45 °C (2 h) (2 h)	+, −	+, −	+++	−	+, −	+	+	++
	40 °C → 47.5 °C (2 h) (10 min)	+, −	+, −	++	−	+, −	+	+	++

* − denotes absent; +, − denotes some are present and some are absent; +, ++, and +++ denote degrees of characteristic increment.

Table 2. *The effect of heat shock on cell organelles of meristematic cells in soybean root*

Treatment	Plasmalemma	Endoplasmic reticulum	Crest of* mitochondria	Plastid envelope	Nuclear envelope	Nucleolar* fragmentation
28 °C (2 h)	intact	normal	+	intact	intact	−
40 °C (2 h)	intact	swelling	+, −	intact	intact	+
45 °C (2 h)	broken	broken	−	broken	broken	−
47.5 °C (10 min)	broken	broken	+, −	broken	broken	+, −
40 °C → 45 °C (2 h) (2 h)	intact	swelling	+, −	intact	intact	+
40 °C → 47.5 °C (2 h) (10 min)	intact	swelling	+, −	intact	intact	+

* + denotes present, +, − denotes some are present and some are absent.

3.4. 40 °C (2 h) → 45 °C (2 h)

Varying percentages of plasmolyzed cells were found in different meristem tissues (Figs. 2*I* and *J*). Almost all cells in the protoderm and ground meristem were plasmolyzed and their stainability was much greater than that of normal living cells (28 °C). Smaller cells in the peripheral procambium seemed quite normal and only 10% of the cells were plasmolyzed. However, larger cells in the central procambium were less stable, and almost 50% of them were plasmolyzed (Table 1). Cytoplasmic coagulation occurred in the protoderm, in the outer half of the ground meristem, and in the central procambium. But the inner ground meristem and peripheral procambium (except for the potential sieve and vessel cells) were relatively normal.

As shown in Figs. 3*D* and 4*D*, the membranes of the plasmalemma, ER, nuclear envelope, and plastid were intact. Vacuolar fusion of small vacuoles was frequently observed. The general appearance of cells in the peripheral procambium was quite similar to that of cells growing at 28 °C. However, several structural changes are worth mentioning. First, the entire lumen of the ER cisternae showed increasing space (Fig. 4*D*, arrow). Second, there were much fewer crests in the mitochondria when compared to cells grown at 28 °C; some mitochondria were broken (Fig. 4*D*, arrowhead). Third, nucleolar changes were characterized by: 1. An increase in the size of the nucleolus; 2. Vacuolation in the nucleolus; 3. Fragmentation of the nucleolus into a loose, lobe-shaped structure; 4. Segregation of the fibril zone and the granular zone; the granular zones were loosely dispersed in the peripheral regions of the nucleolus (Fig. 3*D*).

3.5. 47.5 °C (10 min)

The damage caused by heat shock was related to the degree of the rise in temperature and the duration of the treatment. Root tip incubation at 47.5 °C for 10 min resulted in root death (Figs. 2*G* and *H*). The plasmolysis, cytoplasmic coagulation, membrane breakage and nucleolus expansion were similar to the results of the test at 45 °C (2 h). However, the meristematic cells showed much less leakage of cell content (Figs. 2*G* and *H*). Unlike the root cells incubated at 45 °C (2 h) which were often uniform in appearance (Fig. 2*F*), varying tissues subjected to a brief shock heat treatment were not uniform in appearance and the procambial cells often released a large oil drop (Fig. 2*H*). As shown in Fig. 4*E*, procambial cells subjected to heat shock at 47.5 °C for 10 min had irregularities in the plasmalemma (arrow) and in the curl of mitochondria membrane (arrowhead). The plastid envelope disappeared and there was nucleolar fragmentation (Fig. 4*E*).

3.6. 40 °C (2 h) → 47.5 °C (10 min)

As shown in Figs. 2*K* and 2*L*, the gross structures of the protoderm and the ground meristem of roots treated at 40 °C (2 h) → 47.5 °C (10 min) were similar to that of cells treated at 40 °C (2 h) → 45 °C (2 h). Almost all cells in both meristems were plasmolyzed; the increase in stainability of the cytoplasm and the number of starch grains were common characteristics of roots treated at 40 °C (2 h) → 47.5 °C (10 min). Most cells in the central region of the procambium were quite normal and had a darkly stained cytoplasm; most cells of the peripheral cambium were plasmolyzed. All cells showed an increase in nucleolar volume, vacuolation of the nucleolus, and fragmentation of the nucleolus. However, the membrane system was intact. As described in Table 2 and in Figs. 3*F* and 4*F*, there was swelling of the cisternae in the ER (Fig. 4*F*, arrow), the number of crests in most mitochondria were basically the same, the ameboid plastid was well-preserved, and vacuolar fusion was found in the procambial cells (Fig. 3*F*).

4. Discussion

Different root tissues react in different ways to optimal and lethal heat shock temperatures. Our preliminary studies of heat shock and its effects upon the growth of different regions of the root showed that the region 1.2 mm from the root tip, which corresponds to subapical meristematic regions was most sensitive (unpublished data). Even in the meristematic regions, different meristems and different sites of the same meristem responded in different ways; this was reflected by changes in stainability, irreversible plasmolysis, cytoplasmic coagulation, membrane breakage, and changes in the reflex index, etc. (reviewed in LEVITT 1980). Irrespective of the various lethal and sublethal heat treatments, protodermal cells always showed plasmolysis and cytoplasmic coagulation. Procambial cells not only reacted differently to heat, but also had site-specific heat tolerance.

An increase in the stainability of the cytoplasm occurred in heat shocked roots, but the structural integrality of the cytoplasm in cells incubated at 45 °C (2 h) and 47.5 °C (10 min) was much different from that of cells treated at 40 °C (2 h), or with a pretreatment at 40 °C for 2 h. The increase in stainability may be due to protoplasmic contraction (LEVITT 1980). However, protoplasmic contraction, instead of angular plasmolysis, occurred in cells preincubated at 40 °C. This indicated that a certain percentage of root cells died and this resulted in a comparative decrease in the rate of root elongation (LIN *et al.* 1984).

The liberation of lipids as lipid vesicles in cells treated at 47.5 °C (10 min) could be the primary injury caused by heat treatment that results in cell death; roots incubated at 45 °C (2 h) were injured in a different manner. The liquefaction of lipids had been observed in wheat roots (SKOGQVIST 1974), in *Tetrahymena* (ERWIN 1970), and in other species (LEVITT 1980). Most lipids released contained relatively high amounts of unsaturated fatty acids, and this often resulted in cell membrane deformation, which could be reversed by adding polyunsaturated fatty acids (ERWIN 1970). Changes in the composition of lipids in the membrane systems after heat shock let to disruption of the biomembrane (LEVITT 1980); this also affected the conformation of annulus lipids surrounding the membrane protein, which led to decreases of enzyme activity and the capability of the receptors (HOUSLAY and STANLEY 1982). The relationship between lipid composition and incubation temperature has been observed in microorganisms (HUANG *et al.* 1974), alga (KLEINSCHMIDT and MCMAHON 1970), fungi (MUMMA *et al.* 1970) and higher plants (PEARCY *et al.* 1977). At a high temperature, the total lipid content decreased up to one half, and the ratio of unsaturated to saturated fatty acids also decreased up to three times. No lipid liberation was found in roots incubated at 45 °C (2 h), but root growth was greatly retarded (unpublished data). The above facts indicated that the degree of cell injury was related to temperature and the duration of the treatment. Heat damage caused changes and resulted in the loss of the membrane's semipermeability (LEVITT 1980). Heat injured membranes were observed in the plasmalemma (WU and WALLER 1983), nuclear envelope (FELDHERR 1973), chloroplast (BERRY *et al.* 1975), ER, Golgi comples (SKOGQVIST 1975). In the present study, as shown in Fig. 3 and Table 2, when incubated at 45 °C (2 h), all cell organelles collapsed and the membrane systems were disorganized. Nevertheless, cells reacted to heat treatment at 47.5 °C (10 min) in a different manner: the plasmalemma were broken: the ER membrane, the inner membrane of the mitochondria, and the inner membrane of the chloroplast were all disappeared; and the chloroplast envelope, mitochondrial outer membrane, nuclear envelope, and tonoplast were also broken. According to the above observations the greatest thermostability of cell membrane systems was in the plasmalemma, and the tonoplast, followed in decreasing order by plastid envelope, endocytoplasmic membranes (including ER, Golgi and microbodies), crest of mitochondria, and the thylakoid of plastid.

It has been suggested that the nucleus is one part of the cell most sensitive to heat-stimuli (LEVITT 1980). BARABAL'CHUK and CHERNYAVSKAYA (1975) found that the nuclear volume of the *Tradescantia* decreased by 15% at 50 °C (5 min) and decreased by up to 50% at 59 °C (5 min). A study of light micrographs revealed that the ratio of the nucleolus to the nucleus was greatly increased. This may related to the decrease of nuclear volume at lethal temperatures (45 °C (2 h), 47.5 °C (10 min)), and even with a pretreatment at 40 °C. Another possible reason was the increase of nucleolar volume. Electron micrography revealed that nuclear volume did decrease, and that nucleolar volume did not increase significantly. The studies of EDTA counter staining and autoradiography done by SIMARD and BERNHARD (1967) found that nucleolar lesions occurred in BHK cells cultured at 45 °C (10 min) recovery to the original state occurred at 37 °C. They pointed out that there is a heat-sensitive cellular organelle located in the nucleolus. Later studies of the nucleolus and its response to heat shock showed fragmentation

of the nucleoli (DUPRAT 1969) and nucleolar segregation (RISUEÑO *et al.* 1973). Heat-induced nucleolar segregation was similar to that of cells treated with actinomycin D (GHOSH 1976). This indicated that ribosomal RNA synthesis was temporarily inhibited. As shown in Figs. 3*B* through 3*F*, meristematic cells all showed nucleolar segregation, and granular ribonucleoproteins were dispersed into the nucleoplasm. It can be predicted that fragments of the nucleolus will recover to form compact and solid nucleoli after the plants are returned to a temperature of 28 °C.

Observations made in 1940 of *Spirogyra* of its response to heat showed that swelling of the starch grains occurred in the chloroplast (cited in LEVITT 1980). Meristematic cells subjected to lethal and optimal heat shock temperatures all contained aggregated starch grains. Ultrastructural observations showed that there were several grains within the plastids. As shown in Figs. 4*C* and *F*, the starch grains stopped swelling after the collapse of the plastids.

Soybean seedlings preincubated at 40 °C (2 h) were thermotolerant to lethal temperature treatments of 45 °C (2 h) or 47.5 °C (10 min). The intactness found in structures of plasmalemma, mitochondria, plastids and nuclei may be due to associations of heat shock proteins and is in consistent with the results of localization studies of heat shock proteins during heat shock (ARRIGE *et al.* 1980, CHOU and LIN 1987, HUGHES and AUGUST 1982, LIN *et al.* 1984, LIN *et al.* 1985, VIERLING and KEY 1985).

References

ALEXANDROV VY (1964) Cytophysiological and cytoecological investigations of heat resistance of plant cells toward the action of higher and low temperature. Q Rev Biol 29: 35–77

ARRIGO AP, FAKAN S, TISSIERES A (1980) Localization of the heat-shock-induced proteins in *Drosophila melanogaster* tissue culture cells. Dev Biol 78: 86–103

BARABAL'CHUK KA, CHERNYAVSKAYA VN (1975) Effect of high temperature on nuclear volume in spiderwort leaf cells. Tsitologiya 17: 1223–1226

BAUSER H, SENGER M (1979) Photosynthesis of ivy leaves (*Hedera helix* L.) after heat stress. II. Activities of RuBP carboxylase, Hill reaction and chloroplast ultrastructure. Z Pflanzenphysiol 95: 359–369

BERRY J, BJÖRKMAN O (1980) Photosynthetic response and adaptation to temperature in higher plants. Ann Rev Plant Physiol 31: 491–543

– FORK DC, GARRISON S (1975) Mechanistic studies of thermal damage to leaves. Carnegie Inst Washington Yearb 74: 751–759

CHOU M, LIN CY (1987) Thermotolerance of isolated mitochondria associated with heat shock proteins. Plant Physiol 83 (4): 70s

DANIELL JW, CHAPPELL WE, COUCH HB (1969) Effect of sublethal and lethal temperature plant cells. Plant Physiol 44: 1684–1689

DANGEARD P (1951) Observations sur la destruction du chrondriome par la chaleur. C R Hebd Seances Acad Sci 232: 1274–1276

DAS PK (1973) Developmental stability and thermosensitivity of different varieties of wheat. Nucleus 16: 175–179

DUPRAT NA (1969) Effects de temperatures supra-normales sur le nucleole de cellules embryonnaires durodeles en culture in vitro. Expt Cell Res 57: 37–42

ERWIN JA (1970) The reversal of temperature-induced cell surface deformation in *Tetrahymena* by polyunsaturated fatty acid. Biochim Biophys Acta 202: 21–34

FELDHERR CM (1973) The effect of temperature on nuclear permeability. Experimentia 29: 546–547

GHOSH S (1976) The nucleolar structure. Internat Rev Cytol 44: 1–28

HOUSLAY MD, STANLEY KK (1982) Dynamics of biological membrane. John Wiley & Sons, Chichester.

HUANG L, LORCH SK, SMITH GG, HUAG A (1974) Control of membrane lipid fluidity in *Acholeplasma laidlawii*. FEBS Lett 43: 1–5

HUGHES EN, AUGUST JT (1982) Coprecipitation of heat shock protein with a cell surface glycoprotein. Proc Natl Acad Sci USA 79: 2305–2309

KLEINSCHMIDT MG, MCMAHON VA (1970) Effect of growth temperature on the lipid composition of *Cyanidium calforium* I. Class separation of lipids. Plant Physiol 46: 286–289

KRAUSE GH, SANTARIOUS DA (1975) Relative thermostability of the chloroplast envelope. Planta 127: 285–299

LEVITT L (1980) Responses of plants to environmental stress, Vol I. Chilling, freezing and high temperature stress. Academic Press, New York.

LIN CY, ROBERT JK, KEY JL (1984) Acquisition of thermotolerance in soybean seedling: synthesis and accumulation of heat shock proteins and their cellular localization. Plant Physiol 74: 152–160

– CHEN YM, KEY JL (1985) Solute leakage in soybean seedlings under various heat shock regines. Plant Cell Physiol 26: 1493–1498

MUMMA RO, FERGUS CL, SEKURA RS (1970) The lipids of thermophilic fungi: lipid composition comparisons between thermophilic and mesophilic fungi. Lipids 5: 100–103

PEARCY RW, BERRY JA, FORK DC (1977) Effect of growth temperature on thermal stability of the photosynthetic apparatus of *Atriplex lentiformis* (Torr) Wats. Plant Physiol 59: 873–878

RISUEÑO MC, STOCKET JC, GIMÉNEZ MC, DIEZ JL (1973) Effect of supraoptimal temperature on meristematic cell nucleoli. J Microsc (Paris) 16: 87–94

SCHAEFERS HA, FEIERABEND J (1976) Ultrastructural differentiation of plastids and other organelles in rye leaves with high-temperature induced deficiency of plant ribosomes. Cytobiologie 14: 75–90

SIMARD R, BERNHARD W (1967) A heat-sensitive cellular function located in the nucleolus. J Cell Biol 34: 61–76

SKOGQVIST I (1974) Induction of heat sensitivity of wheat roots and its effects on mitochondria, adenosine triphosphate, triglyceride and total lipid content. Expt Cell Res 86: 285–294

SPURR RA (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. J Ultrastruct Res 26: 31–43

VIERLING E, KEY JL (1985) Ribulose-1,5-bisphosphate carboxylase synthesis during heat shock. Plant Physiol 78: 155–162

WU MT, WALLER SJ (1983) Heat stress responses in cultured plant cells: development and comparison on vitality test. Plant Physiol 72: 817–820