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Cytology of potato callus cells in relation to their frost hardiness

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Abstract

Structure of callus cells of frost-sensitive and frost-tolerant *Solanum* species and a frost-tolerant cell line (D20-1), selected from *S. tuberosum* cv. Desirée callus, was studied. Like frost-tolerant species *S. commersonii*, cells of the frost-tolerant cell line contained starch grains in their plastids. The cells of this frost-tolerant line also possessed an increased number of microbodies containing protein crystals which suggests the involvement of proteins in frost tolerance but the mechanism may differ from that in frost-tolerant species.

Additional key words: frost tolerance, microbodies, proteins, *Solanum*.

Introduction

Many crop plants are cold-sensitive and usually are damaged or killed when exposed to freezing temperature. Commercially cultivated potato (*Solanum tuberosum* L.) is killed at temperature -2 to -3 °C even after hardening, while a number of non-cultivated, tuber-bearing *Solanum* species, *e.g.*, *S. commersonii* and *S. acaule* can survive -4.5 and -6 °C, respectively. Among these frost-tolerant species, some are also able to cold acclimate (Irzykowski *et al.* 1996). *S. commersonii* and *S. acaule* are cabable to cold acclimate and they withstand temperatures as low as -9 and -11.5 °C, respectively when exposed to 2 °C for 20 d (Chen and Li 1980). However, frost tolerance and cold acclimation are genetically distinct traits with independent genetic control (Stone *et al.* 1992, 1993).

Low temperature tolerance may involve cytoplasmic factors (Sutka *et al.* 1991) and trait may be inherited maternally (Handley and Sink 1985). It is possible that the differences in frost tolerance among *Solanum* species may correlate with the nature and organization of their cellular structure. In the present study, cellular structure of callus cells was investigated to compare ultrastructural differences between frost-sensitive (*S. tuberosum* cv. Desirée) and frost-tolerant species (*S. commersonii* and *S. acaule*), and a frost-tolerant cell line (D20-1), with frost killing temperature of -4.2 °C, selected from callus cultures of *S. tuberosum* cv. Desirée (Anjum and Villiers 1998).

Materials and methods

Callus was initiated from leaf pieces of *S. tuberosum* (cv. Desirée), *S. acaule* and *S. commersonii* and maintained on MS medium (Murashige and Skoog 1962) supplemented with 3 mg dm^{-3} 2,4-D, 0.3 mg dm^{-3} kinetin, 30 g dm^{-3} sucrose and 8 g dm^{-3} agar. A frost-tolerant cell line (D20-1), selected from the callus cultures of *S. tuberosum* (Anjum and Villiers 1998) was also used for the study. All the calli were about six months old and specimens were prepared from the actively growing calli (10 d after subculture) for observation by electron microscopy in the following way.

The double fixative glutaraldehyde-osmium (Juniper *et al.* 1970) was used for the fixation of callus cells.

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2.5 % glutaraldehyde was prepared in 0.1 M Na-cacodylate buffer (pH 7.2). Callus was trimmed into cubes (2 mm^3) and these were transferred to a specimen tube containing glutaraldehyde. After 1.5 h, the fixative was removed by three washings (0.1 M Na-cacodylate buffer, 5 min each). Tissues were then post-fixed in 1 % osmium tetroxide in 0.1 M Na-cacodylate buffer for about 2 h. Then three washings of buffer were given each for 5 min to remove all the traces of fixative from the tissues. Specimens were dehydrated through a series of 30, 50, 70 and 90 % acetone at room temperature, each change for 5 min followed by 5 changes of absolute acetone.

Epoxy resin, used for embedding the specimens, was prepared by mixing 40 g agar 100, 22 g *Araldite CY212*, 72 g dodecenyl succinic anhydride and 2 g benzyl-dimethylamine (Mollenhauer 1964). Absolute acetone was replaced by 1:1 acetone/resin mixture and specimens were placed on a slowly rotating machine to assist penetration of resin mixture into the tissue. After 1 h the acetone/resin mixture was replaced by fresh resin mixture and the specimens rotated overnight. Then each specimen was placed at the bottom of a *BEEM* capsule, which was filled with fresh resin and placed in an oven at 60 °C overnight.

Blocks were trimmed by a razor to expose the specimen in an orientation to present a suitable face to the knife. Glass knives were made by *LKB Knifemaker* just before cutting the sections. Sections were cut by an *LKB Ultratome III* and floated onto water held behind the cutting edge with a strip of PVC tape. The sections were then mounted on 3.05 mm copper mesh grids for viewing in the electron microscope. Only silver and gold-coloured sections were selected.

Grids with sections were washed with distilled water and stained for 30 min in uranyl acetate, after which the grids were again washed with distilled water and stained with lead citrate for 5 min (Reynolds 1963). Specimens were again washed with distilled water and dried. Finally, the stained sections were observed in a *Cornith 500* transmission electron microscope and selected areas were photographed.

Results and discussion

Callus cells of *S. tuberosum* cv. Desirée contain very large vacuoles (Fig. 1*A*) occupying major volume of the cells. A few vesicles can also be seen at the surface of the tonoplast apparently passing into the vacuole. The nucleus lies to one side of the cell surrounded by a thin layer of cytoplasm. The size of nucleus appears quite large with usually one nucleolus in view, while the shape appears quite variable, from a simple sphere to many-lobed (Fig. 1*B*) depending upon the plane through which the sections were cut. The nucleus is bound by the nuclear envelope comprised of two unit membranes. Most of the nuclear volume is occupied by chromatin. The shape of the nucleolus is usually spherical but in the nucleus of a cell, a lobe-shaped nucleolus can be seen (Fig. 1*B*). The nucleolus contains one or more electron-transparent regions, a dense granular zone and also an area with very lightly staining properties (Fig. 1*B*) which might be nucleolar vacuole, regarded as a sign of an active nucleolus. In the Fig. 1*B*, nucleolar organizer can be seen, as the place of connection with the chromatin material.

The cytoplasm of cell, enclosed by a plasma membrane and surrounded by the cell wall contains different types of organelles. Among these plastids, mitochondria and microbodies are most prominent (Figs. 1*A,B*). Plastids are usually bigger than mitochondria. The shape of the mitochondria may appear circular or elliptical according to the plane of the section. Some microbodies or peroxisomes, containing crystalline granules, are also present in the cytoplasm (Figs. 1*A,B*), which are probably of proteins and are commonly found in the cells of storage tissue (Burgess 1985). In the cytoplasm, the paired membrane sheets (endoplasmic reticulum) can also be seen (Fig. 1*B*).

The callus cells of *S. commersonii* (a frost-tolerant species) present a similar structure with a complete range of cellular organelles (Figs. 2*A,B*). The most prominent cellular organelles, after the nucleus, are the plastids and mitochondria. Some plastids, usually larger in size, also contain starch grains, which are darkly stained in the photographs (Fig. 2*A*). The cells of *S. acaule* (also a frost-tolerant species) appear to contain more cytoplasmic contents compared with cv. Desirée callus cells possibly because the callus cells are smaller, or the callus is younger. Organelles such as plastids and mitochondria are confined to the periphery of the cytoplasm (Figs. 3*A,B*). Besides regular and cup-shaped plastids, some unusual-shaped plastids are also present, while endoplasmic reticulum appears to be swollen (Fig. 3*A*). Microbodies containing protein crystals and dictyosomes can also be seen in the cytoplasm (Fig. 3*B*). It is clear that the callus cells of *Solanum* species possessed a normal range of cellular organelles, and their structure and arrangement suggested that the cells were metabolically active. In the cells of *S. tuberosum* and *S. acaule*, very little starch was seen in the plastids, and this suggests that the cells were fixed in an active state of growth.

The callus cells of frost-tolerant line (D20-1) appear to contain an increased number of microbodies containing protein crystals in the cytoplasm (Figs. 4*A,B*). While

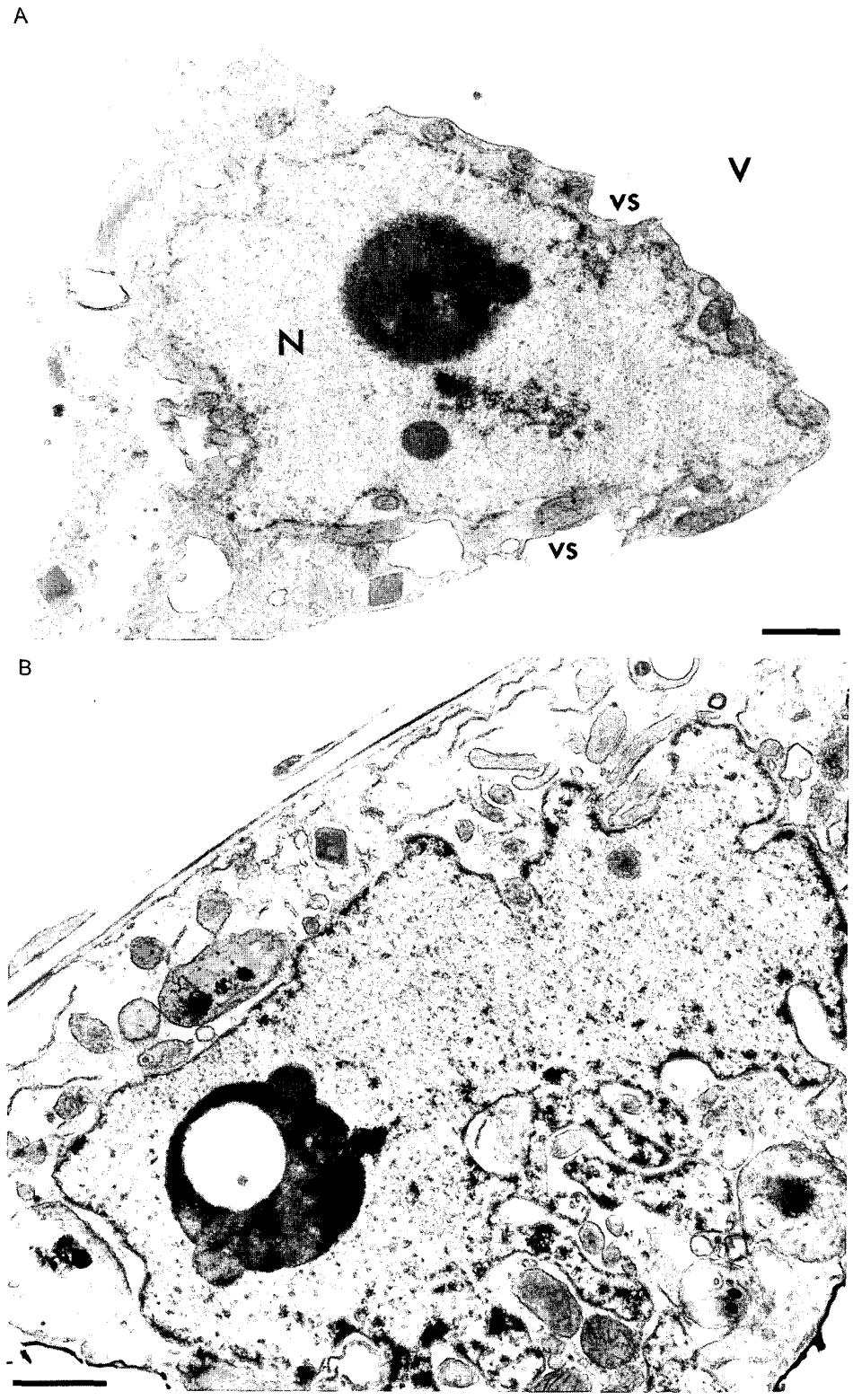

Fig. 1. *S. tuberosum* cv. Desirée: *A* - part of a cell showing nucleus (N) surrounded by a thin layer of cytoplasm containing plastids, mitochondria and microbodies containing protein crystals. Vesicles (vs) appear to be discharging into the vacuole (V) (*bar* = 2 μm); *B* - general view of nucleus showing a nucleolus with a lightly stained area, possibly nuclear vacuole and nucleolar organizer. Plastids, mitochondria and microbodies can also be seen in the cytoplasm (*bar* = 2 μm).

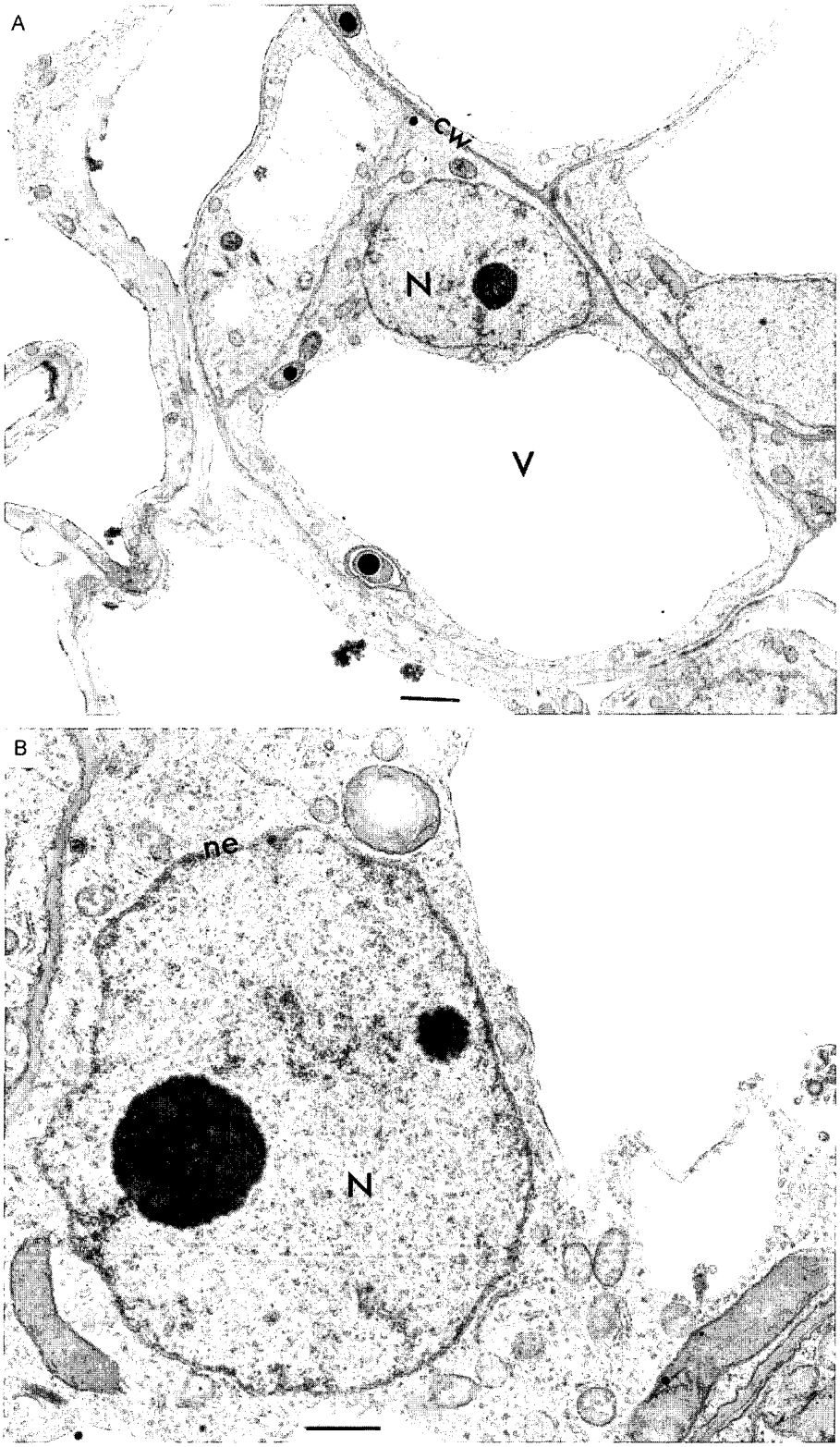

Fig. 2. *S. commersonii*: *A* - cells showing the cell wall (cw), cytoplasm containing different organelles, nucleus (N) with a nucleolus, and vacuole (V) (*bar* = 2 μm); *B* - showing a nucleus (N) with chromatin surrounded by nuclear envelope (ne) and different organelles in the cytoplasm (*bar* = 1 μm).

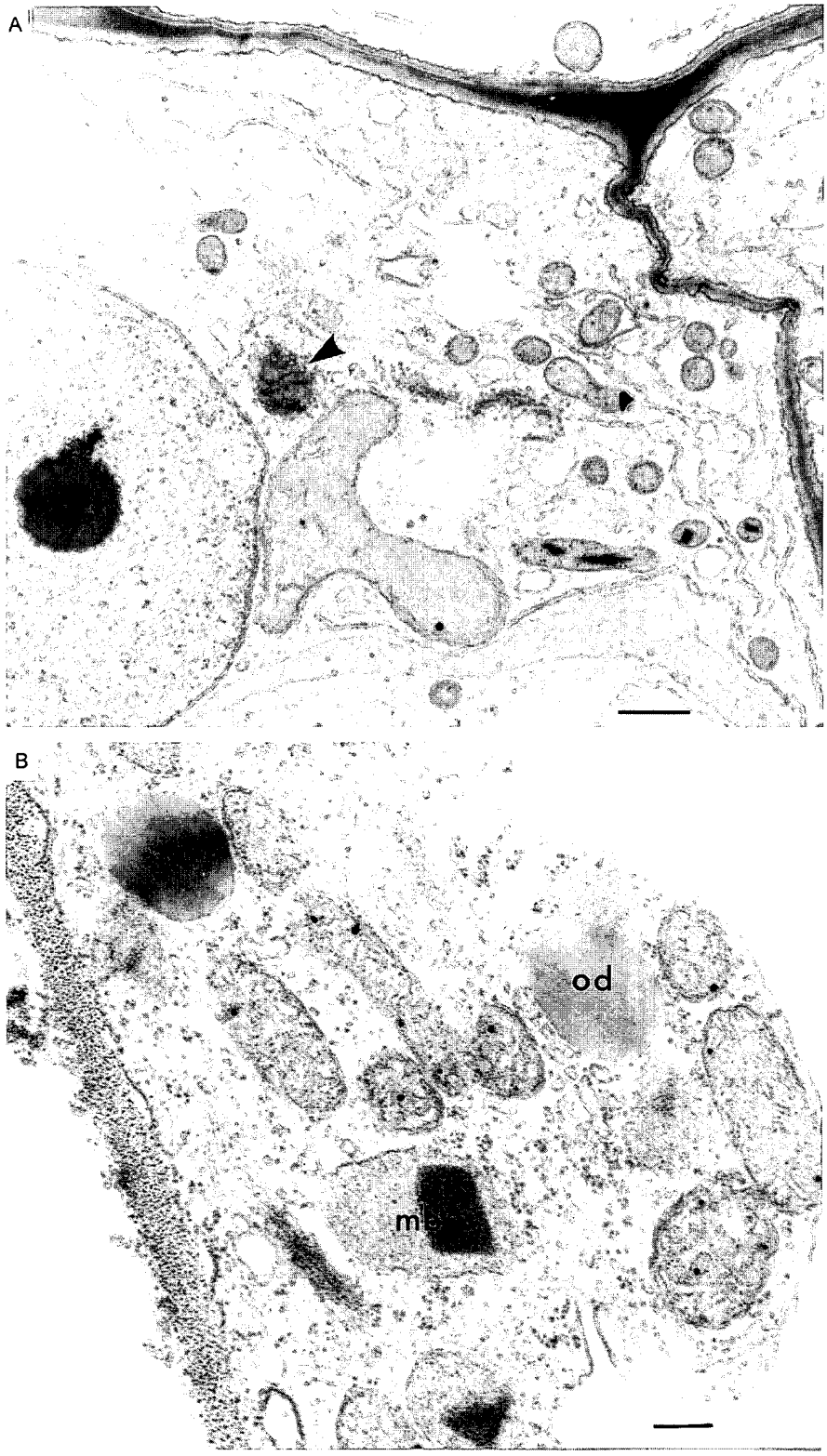

Fig. 3. *S. acaule*: *A* - part of cell showing cytoplasm, surrounded by a cell wall, containing plastids, ribosomes and dictyosomes (arrow), and a nucleus containing a nucleolus with a nucleolar organizer (*bar* = 1 μm); *B* - part of peripheral cytoplasm adjacent to the cell wall, showing the plasma membrane, microbodies containing protein crystals (mb) and oil droplets (od) (*bar* = 0.2 μm).

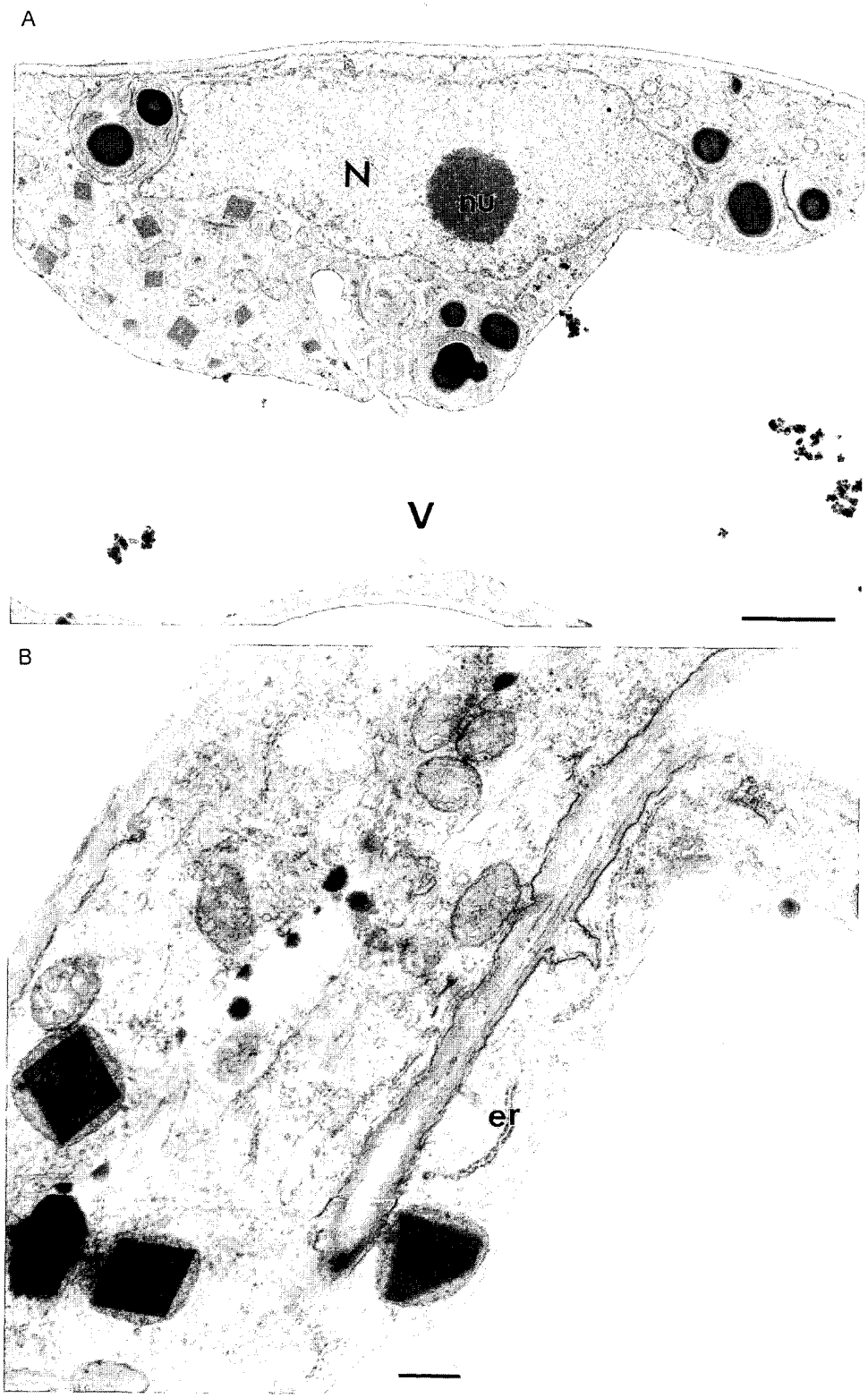

Fig. 4. Frost-tolerant cell line (D20-1): *A* - part of cell showing vacuole (V), nucleus (N) with a nucleolus (nu) and cytoplasm containing large plastids with starch grains, various microbodies with protein crystals and mitochondria (*bar* = 2 μm); *B* - part of cytoplasm showing endoplasmic reticulum (er), microbodies containing protein crystals (mb) and other organelles (*bar* = 0.5 μm).

normal-shaped palstids, many mitochondria and microbodies, and also some large plastids containing starch grains (Fig. 4*A*) can be seen similar to the callus cells of *S. commersonii*. Chen *et al.* (1977) have made attempts to locate ultrastructural differences in the leaf mesophyll cells of frost-sensitive and frost-tolerant *Solanum* species. They reported that the cell walls of leaf mesophyll cells of *S. acaule* were about twice as thick as those of *S. tuberosum* cv. Red Pontiac, and that the chloroplasts of *S. acaule* contained a higher number of osmiophilic globules than the chloroplasts of *S. tuberosum*. These differences could not be demonstrated in the callus cells in the present work, possibly because of their active state of growth. However, callus cells of the frost-tolerant line (D20-1) selected from Desirée callus differed in the starch grains present in the plastids and also in the number of microbodies containing protein crystals from the cells of *S. tuberosum* cv. Desirée and other two *Solanum* species. It is hypothesised that the presence of an increased number of microbodies containing protein crystals suggests the involvement of proteins in frost tolerance. However, as this was not noticed in the callus cells of the frost-tolerant wild species, the mechanism of frost tolerance could be different. Synthesis of new proteins has already been reported in soybean (Cabane *et al.* 1992), winter rye (Antikainen and Pihakaski 1993) and wheat leaves (Pinedo *et al.* 2000) during cold acclimation or when exposed to low temperature. This acclimation-related change in protein patterns is associated with the alterations in expression of specific genes (Rorat *et al.* 1997). Moreover, in the vacuoles of the frost-tolerant callus cells only, some darkly-stained spots were seen in the photographs (Fig. 4*A*) that could be possibly due to substances such as proline, which had been over-produced and/or accumulated in the cell vacuoles.

References

Anjum, M.A., Villiers, T.A.: Selection of hydroxyproline-resistant cell lines from *Solanum tuberosum* L. callus. I. Stability and frost tolerance. - J. Genet. Breed. **52**: 113-117, 1998.

Antikainen, M., Pihakaski, S.: Cold-induced changes in the polysome pattern and protein synthesis in winter rye (*Secale cereale*) leaves. - Physiol Plant. **89**: 111-116, 1993.

Burgess, J.: An Introduction to Plant Cell Development. - Cambridge University Press, Cambridge 1985.

Cabane, M., Vincens, P., Boudet, A.M.: Protein synthesis at low temperatures in two soybean cultivars differing in their cold sensitivity. - Physiol. Plant. **85**: 573-580, 1992.

Chen, H.H., Li, P.H.: Characteristics of cold acclimation and deacclimation in tuber-bearing *Solanum* species. - Plant Physiol. **65**: 1146-1148, 1980.

Chen, P., Li, P.H., Cunningham, W.P.: Ultrastructural differences in leaf cells of some *Solanum* species in relation to their frost hardiness. - Bot. Gaz. **138**: 276-285, 1977.

Handley, L.W., Sink, K.C.: Plant regeneration of protoplasts isolated from suspension cultures of *Solanum lycopersicoides*. - Plant Sci. **42**: 201-207, 1985.

Irzykowski, W., Rorat, T., Jakubiec, J.: Analysis of cold resistance in wild and cultivated potato species. - Acta Physiol. Plant. **18**: 217-221, 1996.

Juniper, B.E., Cox, G.C., Gilchrist, A.J., Williams, R.R.: Techniques for Plant Electron Microscopy. - Blackwell Scientific Publications, Oxford 1970.

Mollenhauer, H.H.: Plastic embedding mixture for use in electron microscopy. - Stain Technol. **39**: 111-114, 1964.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tobacco tissue cultures. - Physiol. Plant. **15**: 473-479, 1962.

Pinedo, M.L., Hernández, G.F., Conde, R.D., Tognetti, J.A.: Effect of low temperature on the protein metabolism of wheat leaves. - Biol. Plant. **43**: 363-367, 2000.

Reynolds, E.S.: The use of lead citrate at high pH as an electron opaque stain in electron microscopy. - J. Cell Biol. **17**: 208-213, 1963.

Rorat, T., Irzykowski, W., Grygorowicz, W.J.: Identification and expression of novel cold induced genes in potato (*Solanum sogarandinum*). - Plant Sci. **124**: 69-78, 1997.

Stone, J.M., Palta, J.P., Bamberg, J.B., Weiss, L.S.: Freezing tolerance and cold acclimation capacity are genetically distinct quantitative traits in a segregating *Solanum* population. - Plant Physiol. **99** (Suppl.): S-126, 1992.

Stone, J.M., Palta, J.P., Bamberg, J.B., Weiss, L.S., Harbage, J.F.: Inheritance of freezing resistance in tuber-bearing *Solanum* species: Evidence for independent genetic control of non-acclimated freezing tolerance and cold acclimation capacity. - Proc. nat. Acad. Sci. USA **90**: 7869-7873, 1993.

Sutka, J., Worland, A.J., Maystrenko, O.I.: Slight effect of cytoplasm on frost resistance in wheat (*Triticum aestivum* L.). - Cereal Res. Commun. **19**: 311-317, 1991.