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Ultrastructural changes in gametophytes of *Acrostichum aureum* L. cultured in different sodium chloride concentrations

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Abstract

Gametophytes cultured in solutions containing 0.0 to 0.7 % NaCl exhibited no change in ultrastructural organization of chloroplasts. In 1.0 % NaCl-grown gametophytes, there were thinner granal stacks, relatively larger spaces between granal thylakoidal membranes and larger plastoglobuli in the chloroplasts. These changes were accompanied by a decrease in photosynthesis. Cup shape, horseshoe shape, ring shape, and amoeboid mitochondria were observed in gametophytes grown in 0.0 to 0.7 % NaCl. Only round mitochondria were observed in the gametophytes grown in 1.0 % NaCl. Mitochondria seemed to be more resistant to salt stress compared to chloroplasts. There was no direct relationship between changes in respiration rate and changes in mitochondrial shape among gametophytes grown in different NaCl concentrations.

Additional key words: chloroplasts, fern, mitochondria, photosynthesis, respiration, salt stress, ultrastructure.

Introduction

Under salt stress the changes in metabolism are usually accompanied by changes in ultrastructural organization of plant cells. When pea plants were treated with 70 mM NaCl, increases in H_2O_2 production and lipid peroxidation were accompanied by the disorganization of cellular membrane, disarrangement of thylakoids and an increase in plastoglobuli in chloroplasts (Hernández *et al.* 1995). In rice, NaCl induced a decrease in net photosynthesis along with the loss of integrity of the chloroplast envelope and the disorganization of grana, while the number of cristae in

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mitochondria was reduced and the cristae were often shortened (Flowers *et al.* 1985). Research on pedunculate oak seedlings showed that mitochondria were less sensitive to salt stress compared with chloroplasts (Schmer *et al.* 1995).

During salt hardening there were also some structural changes. In salinity-adapted tobacco cell cultures, there was a loss of starch grains, an increase in thylakoid membranes and the presence of plastoglobuli in chloroplasts (Locy *et al.* 1996). Photosynthetic O_2 evolution and CO_2 fixation, and photophosphorylation also increased in these tobacco cells. Salt-tolerant embryogenic calli of *Citrus limon* (L.) had thicker cell walls, ring-shape mitochondria and increases in the number of lipid bodies, microbodies and rough endoplasmatic reticulum (Piqueras *et al.* 1994). In meristematic cells of barley roots, salt stress resulted in the production of amoeboid plastids and mitochondria (Huang and Van Stevening 1990, Yan 1995). Salt stress induced membrane vesiculation and tubule formation (Golombek *et al.* 1994, Cachorro *et al.* 1995) in both halophytes and glycophytes; the frequency of pinocytotic vesiculation also increased (Nassery and Jones 1975, Kurkova and Balnokin 1994).

Acrostichum aureum L. is a salt-tolerant mangrove fern. Our research showed that gametophytes of this fern can grow in 0.0 to 1.0 % NaCl solutions. In this paper the ultrastructural organization of gametophytes grown in different NaCl levels were studied and their relationship with respiration and photosynthesis was analyzed.

Materials and methods

Spores of *A. aureum* were collected in Singapore. They were then sterilized with 4 % (v/v) commercial bleach (*Clorox*^{TM}) and sown in one-tenth strength Hoagland solution with different concentrations of NaCl, ranging from 0.0 to 1.0 % (m/v) at a density of two to three thousand spores per Petri dish in 15 cm^3 culture solution. Spore cultures were then kept at 27 °C and at a irradiance of 70 $\mu mol\ m^{-2}\ s^{-1}$ (12 h day/12 h night). Gametophytes at the mature cordate stage (about 5 weeks old) were used for experiments.

Rates of dark respiration and photosynthesis were measured with a leaf-disc oxygen electrode (Walker 1987) and light was provided with *Hansatech LS2* light source (*Hansatech*, Norfolk, UK). The chlorophyll content was determined spectrophotometrically according to Arnon (1949).

The sections of gametophytes (*ca.* 1 mm × 3 mm) were cut and fixed in 1.25 % glutaraldehyde and 1.0 % paraformaldehyde in 50 mM cacodylate buffer (pH 6.8) at room temperature. After rinsing in 50 mM cacodylate buffer 5 times (each time for about 15 min), the samples were post-fixed in 2 % osmium tetraoxide in 50 mM cacodylate buffer for 2 h. The samples were then dehydrated in a graded ethanol series, infiltrated and embedded in freshly prepared Spurr-low viscosity resin and polymerized at 70 °C for 24 h. Ultrathin sections were cut with an ultramicrotome (*Ultracut E, Reichert-Jung*, Wien, Austria), stained with 1 % uranyl acetate for 55 min and lead citrate for 10 min, and then examined using a transmission electron microscope (*Philips CM 10*, Eindhoven, The Netherlands).

Results and discussion

Dark respiration rate and radiation-use efficiency (RUE - slope of linear part of curve expressing response of photosynthetic rate to irradiance) increased with the increase in NaCl concentration (up to 0.7 % NaCl), while light-saturated photosynthetic rate showed no significant changes (Table 1). Both respiration and photosynthesis decreased in gametophytes grown in 1.0 % NaCl solution (Table 1).

Table 1. Changes in respiration rate, photosynthetic rate (measured at saturating irradiance) and RUE of gametophytes grown under different NaCl concentrations.

NaCl concentration [%]	Dark respiration rate [$\mu mol(O_2)\ mg^{-1}(d.m.)$]	RUE [$\mu mol(O_2)\ g^{-1}(Chl)\ \mu mol^{-1}(photon)\ m^2$]	Photosynthetic rate [$\mu mol(O_2)\ g^{-1}(Chl)\ s^{-1}$]
0.0	114 ± 19	0.185 ± 0.028	11.3 ± 0.9
0.2	153 ± 9	0.269 ± 0.019	12.3 ± 0.9
0.5	170 ± 14	0.314 ± 0.013	12.7 ± 0.9
0.7	190 ± 19	0.348 ± 0.025	12.8 ± 0.9
1.0	136 ± 13	0.211 ± 0.017	6.9 ± 0.5

There was no change in the ultrastructure of chloroplasts in gametophytes grown in 0.0 to 0.7 % NaCl solutions. As usual, chloroplasts had both granal and stromal membrane systems, starch grains and small plastoglobuli (Fig. 1*a*). In chloroplasts of gametophytes grown in 1.0 % NaCl, there were smaller stacks of the granal membrane systems and relatively large space were present between granal membranes. Bigger plastoglobuli were also observed (Fig. 1*b*). These ultrastructural changes in chloroplasts could be related to the lower rate of photosynthesis in gametophytes grown in 1.0 % NaCl. But in gametophytes grown in 0.0 to 0.7 % NaCl, the increases in RUE were not correlated to changes in the ultrastructure of chloroplasts.

In gametophytes grown in 0.0 to 0.7 % NaCl, mitochondria of different shapes were observed (Figs. 2*a,b,c*). They included normal round or oval shape mitochondria and mitochondria with abnormal cup shape, horseshoe shape, ring shape, bell shape and amoeboid shape. In amoeboid mitochondria there were some "spaces" with double membranes within the matrix (Fig. 2*c*). There were several reports about these variously shaped mitochondria. All of them were found in meristematic cells of plants (Manton 1961, Yan 1995), in cells of dormant tubers (Bagshaw *et al.* 1969), in cells from actively dividing regions of cultured explants (Bagshaw *et al.* 1969), in somatic embryos of *Brassica napus* (Bey 1995) or salt-tolerant callus cells in tissue culture systems (Piqueras *et al.* 1994). There is no report of these variously shaped mitochondria in well-differentiated green cells. Until now the function of these mitochondria is still unknown. Because they were found in meristematic cells, it was thought that they were actively growing mitochondria (Manton 1961). It was also thought that they might exhibit high metabolic activities because of their high surface area/volume ratio (Yan 1995). In gametophytes grown

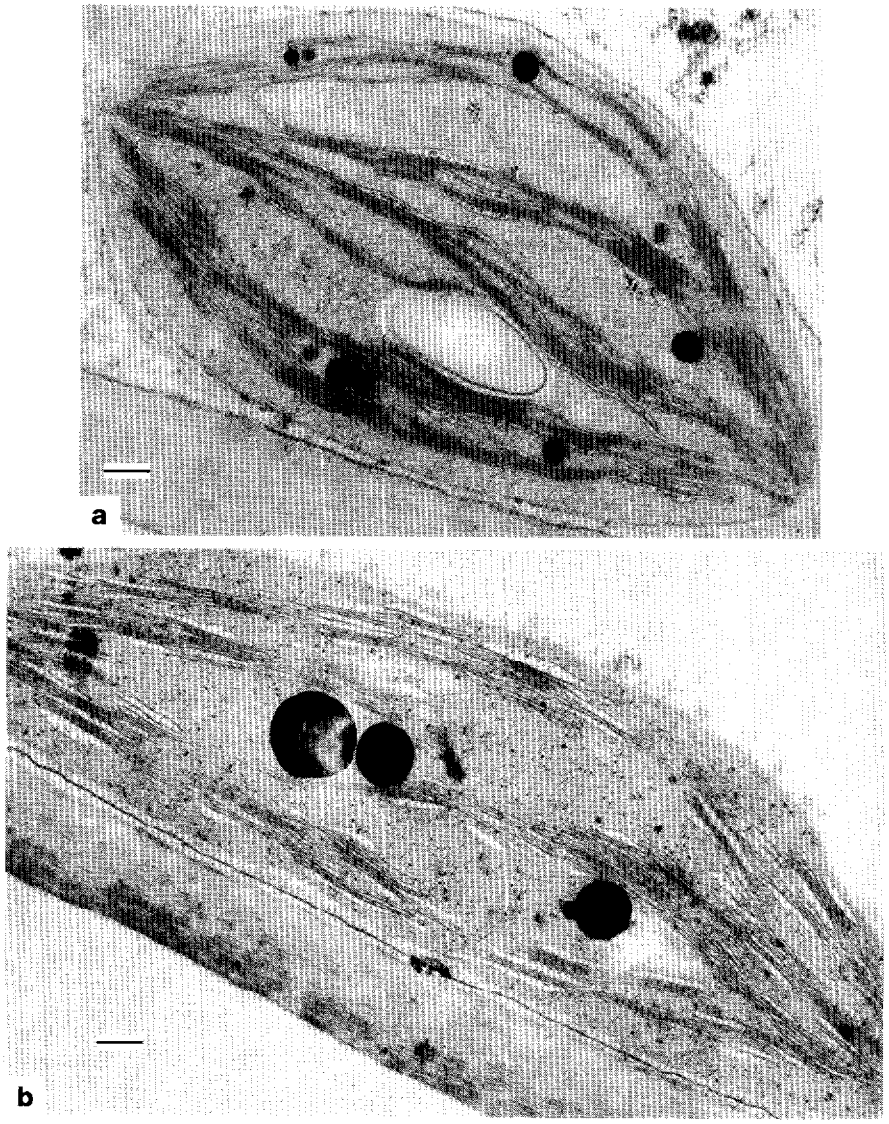

Fig. 1. Chloroplasts in gametophytes grown in 0.0 to 0.7 % NaCl showing normal ultrastructural organization (*a*) and in 1.0 % NaCl showing dilation of granal thylakoids (*b*). *Bar* – 0.2 μm.

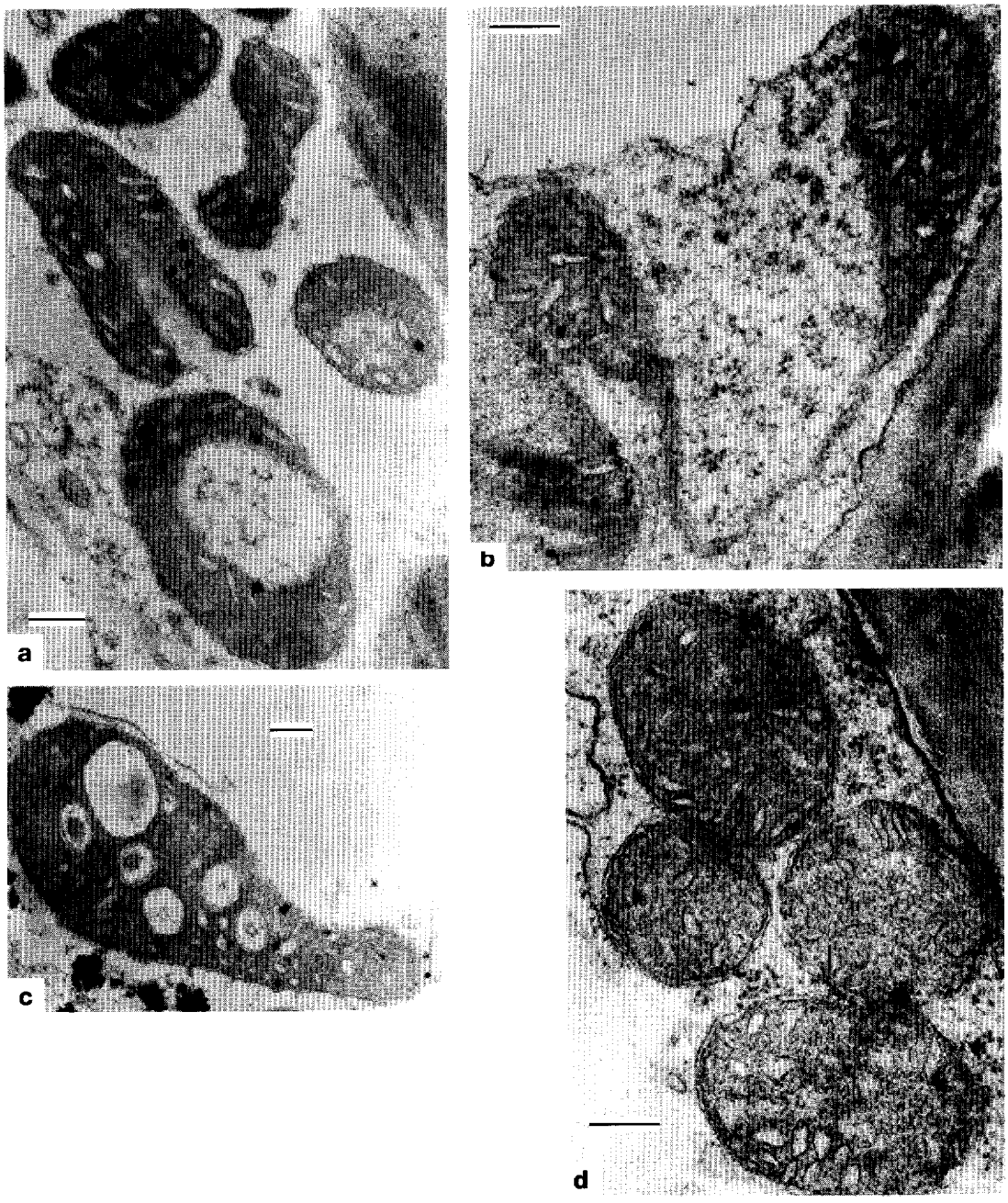

Fig. 2. Differently shaped mitochondria in gametophytes grown in 0.0 to 0.7 % NaCl (*a*, *b*, *c*) and only round shaped mitchondria in 1.0 % NaCl (*d*). *Bar* = 0.2 μm.

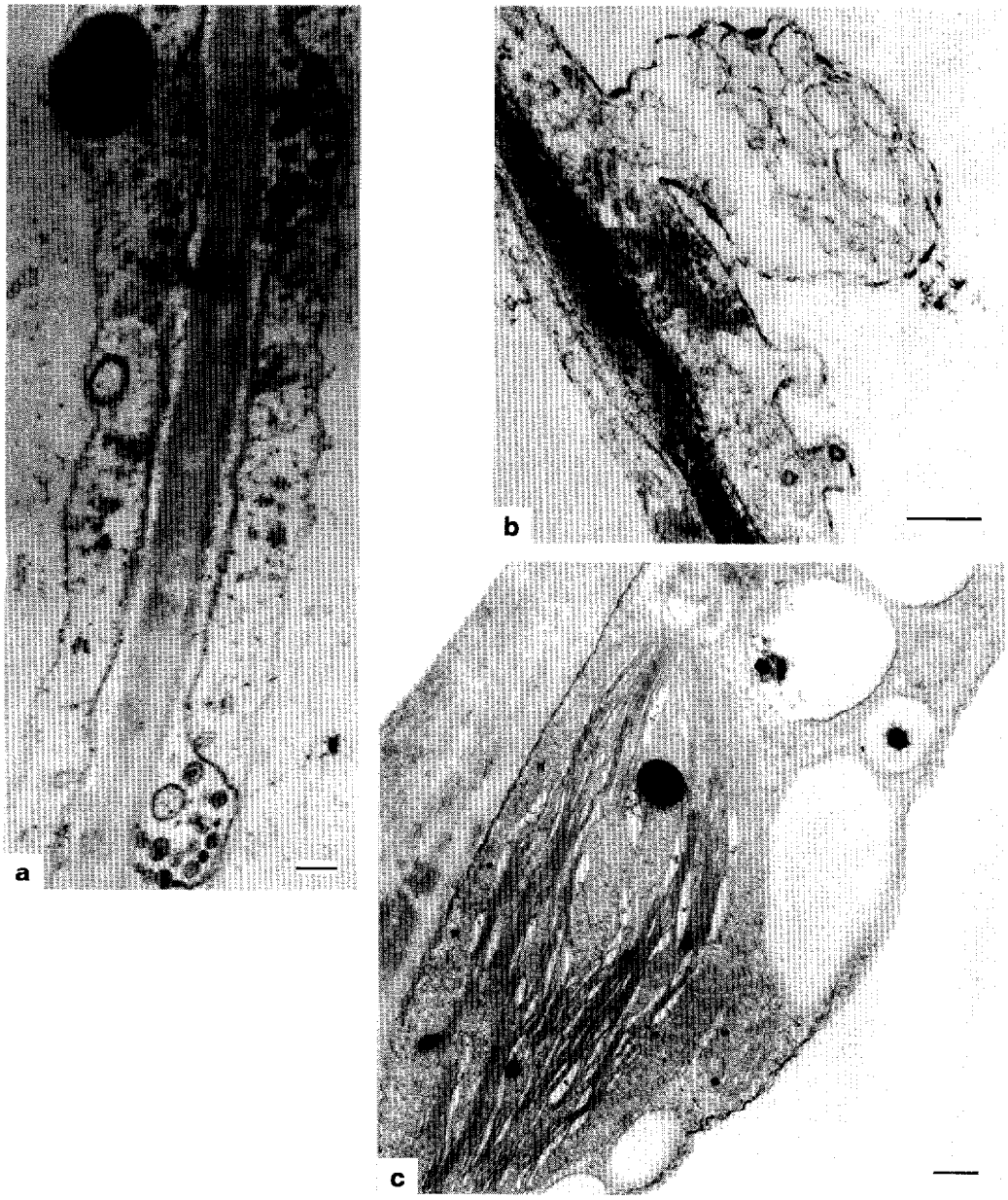

Fig. 3. Possible extrusion of cellular materials found mainly in gametophytes grown in low NaCl and NaCl-free conditions (*a*) and removal of cellular substances into the vacuole observed frequently in gametophytes grown in 0.5 to 1.0 % NaCl (*b*). Vesicles were also commonly found in cytoplasm of gametophytes grown in 1.0 % NaCl (*c*). *Bar* = 0.2 μm.

in 1.0 % NaCl, only round shape mitochondria were observed (Fig. 2*d*). These might be the result of more negative osmotic potential in the cells or a relatively slower growth of gametophytes grown in 1.0 % NaCl. Although there was such a large variation in mitochondrial shape, there seemed to be no difference in the number or the size of cristae among these mitochondria; the membrane system in these mitochondria was not destroyed. However the actual number of mitochondria per cell was not estimated. Respiration rates changed markedly in gametophytes grown at different NaCl levels, but there was no correlation between the shape of mitochondria and the respiration rate. In gametophytes grown in 1.0 % NaCl, the ultrastructural organization of chloroplasts was affected while that of mitochondria seemed not to be affected.

Materials were observed to be extruded from cells in gametophytes grown in all culture conditions (Fig. 3*a*). In gametophytes grown in NaCl solutions more pinocytotic vesiculations were observed (Fig. 3*b*). In gametophytes grown in 1.0 % NaCl a large number of vesicles appeared in the cytoplasm (Fig. 3*c*).

Our study showed that the ultrastructural organization of chloroplasts was more sensitive to salt stress than that of mitochondria. This is in agreement with the results of Flowers *et al.* (1985) and Schmer *et al.* (1995). Similar unusual shape of mitochondria were induced by NaCl in the meristematic cells of barley roots (Yan 1995) or in the salt-adapted cells of calli in *Citrus limon* (Piqueras *et al.* 1994). In gametophytes of *A. aureum* these unusual mitochondria were found in both salt-free and low-salt conditions. Whether these mitochondria could be found in cells of gametophytes at all stages of gametophytic development and persist up to the sporophytic stage, or whether their appearance is influenced by environmental signals, needs futher study.

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