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NaCl-induced amoeboid plastids and mitochondria in meristematic cells of barley roots

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Abstract

The barley (*Hordeum vulgare* L.) seeds were germinated in the non-saline conditions after 12 h imbibition in 2 % of NaCl solution. The results of treatment were: (*1*) the membrane system in meristematic cells of root tips developed well; (*2*) many profiles of endoplasmic reticulum and Golgi bodies appeared; and (*3*) the quantities of amoeboid plastids and amoeboid mitochondria increased. Thus the inhibitory effects of short-term NaCl stress on plants were reversible, and simultaneously NaCl treatment enhanced the metabolic activities in cells. The amoeboid form may be an adaptive form of plastids or mitochondria to an enhanced metabolic activity.

Key words: endoplasmic reticulum, Golgi bodies, *Hordeum vulgare*, imbibition

Introduction

Ultrastructural changes of root cells in response to salt stress have been reported. The main changes are large increases in the quantities of rough endoplasmic reticulum, ribosomes, and mitochondria (Yoe *et al.* 1977), pinocytotic vesiculation in the root of barley and bean (Nassery and Jones 1976), and condensation of chromatin in the nuclei of barley (Werker *et al.* 1983). Huang (1990) found an apparent increase in number of amoeboid plastids in the cortical cells of barley roots under salt stress and regarded the amoeboid plastids as an adaptive change in protein synthesis or composition of the cytoplasm. However, in all the reports mentioned above, the plants were first germinated in a non-saline substrate and then salinized. In fact, effects of salt stress on plants began from the seed imbibition in natural saline environments. In our laboratory, the plants were exposed to a constant salinity during imbibition, and then transferred to the non-saline conditions. The amoeboid plastids and mitochondria appeared in the meristematic cells of the root tips.

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Materials and methods

Seeds of barley (*Hordeum vulgare* L.) were placed in 2 % NaCl solution or distilled water, respectively, each for 12 h in a growth chamber at 25/20 °C (day/night). Seeds were then transferred to aerated 1/4 strength modified Johnson solution (Johnson *et al.* 1957), for 3 d in a growth chamber at 25/20 °C (day/night), with a photoperiod of 14 h and irradiance of 250 μmol m^{-2} s^{-1}.

Segments of root 1 mm from the apex were cut and fixed in 3 % glutaraldehyde in 100 mM phosphate buffer (pH 7.2) for 3 h at room temperature. After two 1-h rinses in the buffer, samples were post-fixed in 1 % osmium tetroxide in the same buffer for 3 h and rinsed two times with the buffer, each for 1 h. The samples were dehydrated in a graded alcohol series, infiltrated and embedded in freshly prepared *Epon 812*, and polymerized at 60 °C for 48 h. Ultrathin sections were cut with a diamond knife, stained with 2 % uranyl acetate for 1 h and with lead citrate for 5 min, and examined using a transmission electron microscope (*JEM-1200 EX, Japan Electronic Company,* Nakagami Akishima Tokyo, Japan).

Results

The membrane system in meristematic cells of root tips developed well during germination after 12 h imbibition in the distilled water, which meant the continuous cytoplasmic membrane, the electron dense plastids and mitochondria with perfect internal cristae (Fig. 1). There was no noticeable changes in the development of the membrane system in meristematic cells of root tips when the barley seeds germinated in the same conditions after 12 h imbibition in 2 % NaCl solution. The cytoplasmic membrane was continuous (Fig. 2), round to oval plastids were full of the high electron dense granules (Fig. 3), and mitochondria had a perfect internal cristae (Fig. 3). However, there were large increases in the quantities of rough endoplasmic reticulum (Fig. 4), Golgi bodies (Fig. 2), amoeboid forms of plastids and mitochondria (Figs. 4 - 12). These amoeboid plastids seemed to be elongated and then their shape was circular. However, the shapes of the plastids were different. Some of the plastids had the shape of horseshoes (Fig. 5) as the middle of them became thin. Some were shaped like a ring (Fig. 8) as two ends of the plastids appeared thin and others were hooks-shaped since one of the two ends seemed thin (Figs. 6, 7). The cytoplasm enclosed by the plastids appeared to be less dense than outside the plastids (Figs. 5, 6, 8), and contained ribosomes (Figs. 5, 6, 8) and mitochondria (Fig. 7).

The amoeboid mitochondria were elongated and became circular, too (Figs. 5, 8, 9, 10, 11, 12). Their shapes were rings (Fig. 8), dumbbells (Fig. 9), and horseshoes (Figs. 5, 10, 11, 12). The cytoplasm enclosed by the amoeboid mitochondria contained ribosomes (Figs. 5, 8), mitochondria (Fig. 11), Golgi body (Fig. 11), and even vacuoles (Fig. 12).

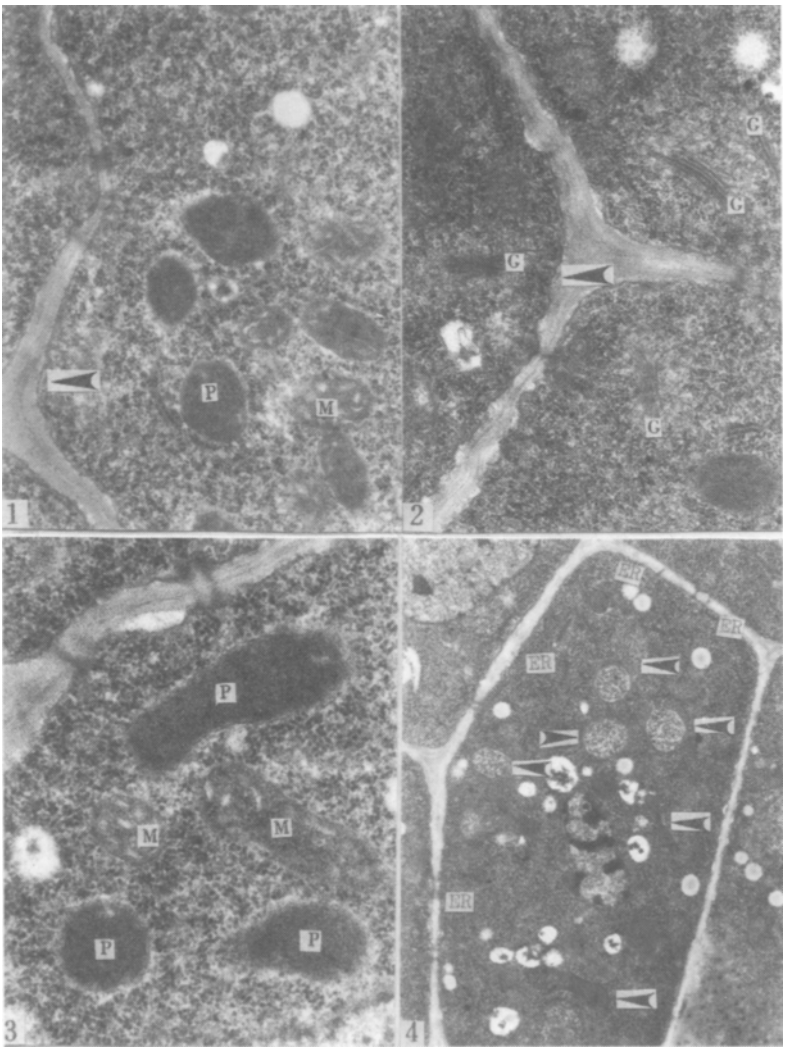


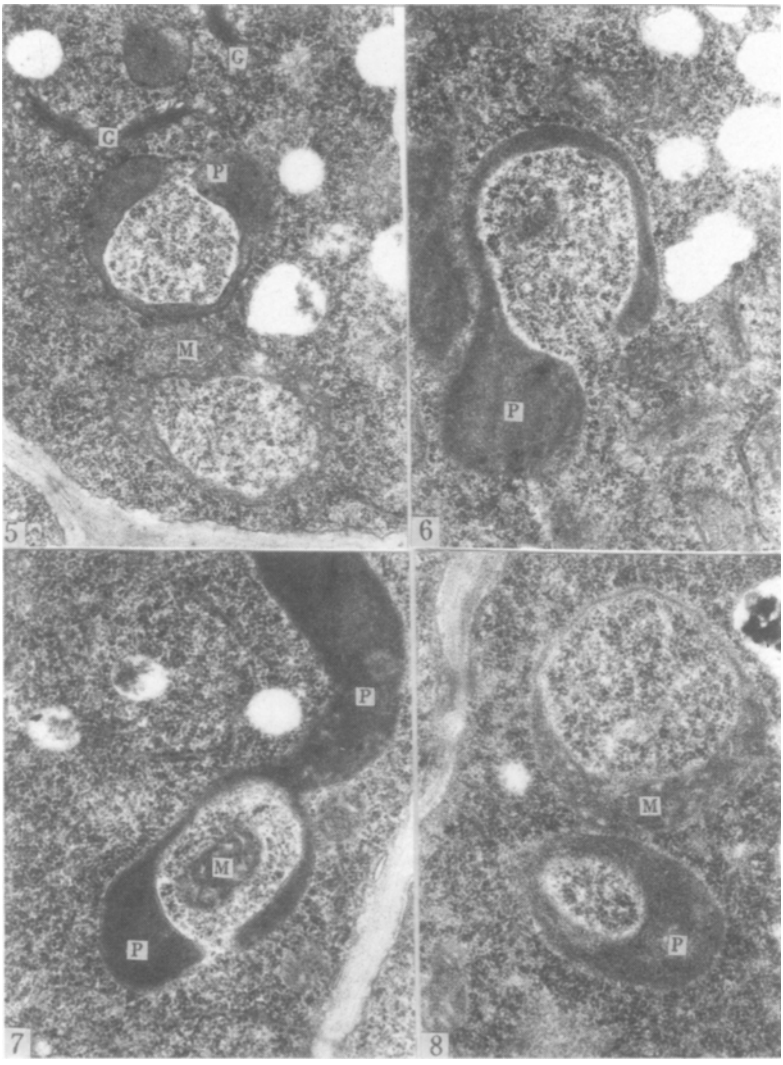
Fig. 1. Ultrastructure of meristematic cells of barley root tips after 12 h imbibition in distilled water showing the round plastids (P), the round mitochondria (M) and the continuous cytoplasmic membrane (*arrow*) (magnification × 20 000).

Figs. 2 - 4. Ultrastructure of meristematic cells of barley root tips after 12 h imbibition in 2 % NaCl solution:

Fig. 2. The continuous cytoplasmic membrane (*arrow*) and many Golgi bodies (G) (magnification × 20 000).

Fig. 3. The round to oval plastids (P) and mitochondria (M) (magnification × 30 000).

Fig. 4. The endoplasmic reticulum (ER) and the amoeboid plastids and mitochondria (*arrow*) (magnification × 6 000).



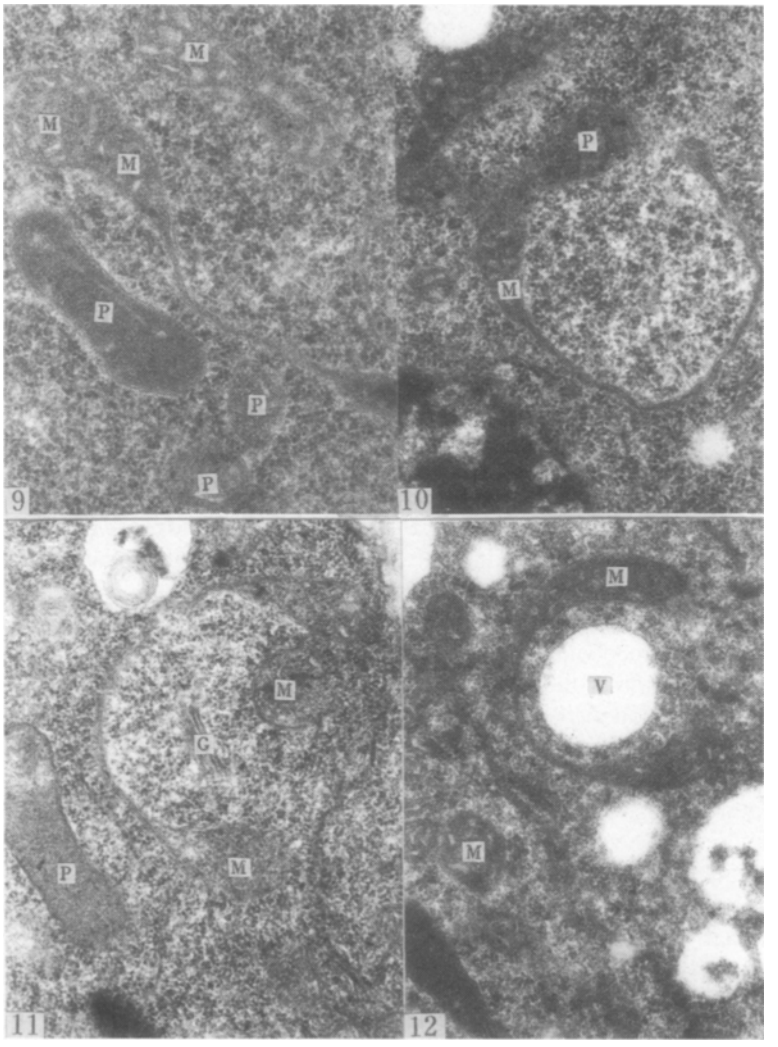
Figs. 5 - 8. Ultrastructure of meristematic cells of barley root tips after 12 h imbibition in 2 % NaCl solution:

Fig. 5. The horseshoe-shaped plastid (P) and mitochondrion (M) and Golgi bodies (G) (magnification × 20 000).

Fig. 6. The hook-shaped plastid (P) (magnification × 20 000).

Fig. 7. The hook-shaped plastid (P) and mitochondrion (M) enclosed by it (magnification × 25 000).

Fig. 8. The ring-shaped plastid (P) and mitochondrion (M) (magnification × 25 000).



Figs. 9 - 12. Ultrastructure of meristematic cells of barley root tips after 12 h imbibition in 2 % NaCl solution:

Fig. 9. The dumbbell-shaped mitochondrion (M) (magnification × 3 000).

Fig. 10. The horseshoe-shaped mitochondrion (M) (magnification × 25 000).

Fig. 11. The horseshoe-shaped mitochondrion (M) and Golgi body (G) and mitochondrion (M) enclosed by it (magnification × 25 000).

Fig. 12. The horseshoe-shaped mitochondrion (M) and vacuole (V) enclosed by it (magnification × 25 000).

Discussion

There have been many reports on the amoeboid plastids. The studies on the development of plastids in the leaves of *Phaseolus vulgaris* and *Zea mays* supposed that the amoeboid form represented the transition of the proplastid from a plastid stage to that of a chloroplast (Whatley 1974, 1977, 1983b, c, Thomson and Whatley 1980). The studies on development of plastids in the roots of *Phaseolus vulgaris* and *Azolla* indicated that the transition was an expression of a spatial sequence of plastid differentiation along the root axis from its tip to more mature regions (Whatley and Gunning 1981, Whatley 1983a). The amoeboid plastids occurred in cells from about 1.5 mm to 4 mm from the junction of the meristematic region and root cap (Whatley 1983a). Other researchers considered that the amoeboid plastids were brought about by environmental factors such as a change in irradiance and salinity (Huang and Van 1990), or internal factors (Weier and Stocking 1962), or the invasion of an actinomycete (Gardner *et al.* 1989). In our experiment, treatment with NaCl during the imbibition induced the increase in number of amoeboid plastids in the meristematic cells of the root tips.

Newcomb (1967) has suggested that the amoeboid shapes may represent a "feeding stage", in which cytoplasmic material was digested and assimilated. Whatley and Whatley (1987) thought that the amoeboid plastids may not only be the equivalent of transfer cells in being able to transport metabolites, but also in being involved in the synthesis of metabolites since these plastids with peripheral invaginations and vesicles have a high surface area/volume ratio. Gardner *et al.* (1989) found the amoeboid type of plastids only within infected cells in the mature nitrogen fixing region of the *Alnus* nodule and speculated that it would indicate that changes in the metabolism of the host cell associated with the onset of nitrogen fixation induced the formation of the amoeboid plastids. Sjolund and Weier (1971) found that in pieces of green light-grown cultures subcultured in a fresh medium a dedifferentiation of the chloroplasts to an amoeboid state occurred at the beginning of cultivation. This reversion of the chloroplasts was paralleled by a dedifferentiation of the vacuolated cells to a less-differentiated meristematic state. In our experiments, the restoration of membrane system was perfect and many profiles of endoplasmic reticulum and Golgi bodies appeared when the seeds were germinated in normal conditions after the NaCl treatment. The results suggested that the inhibitory effect of short-term salt stress on plants was reversible, and simultaneously the metabolic activities in cells were enhanced which induced increase in the quantities of amoeboid plastids.

There has been no report on the amoeboid mitochondria up to now. The amoeboid mitochondria seemed similar to the amoeboid plastids in the shape and the formation. The enhanced metabolic activity induced an increase in the quantities of amoeboid mitochondria, too. The amoeboid mitochondria may exert a high metabolic activity since the amoeboid form with peripheral invagination and vesicles has a high surface area/volume ratio.

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