Ultrastructural changes in rice (Oryza sativa) aleurone cells under NaCl stress

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Ultrastructure of rice aleurone cells after 120 hr of germination has been studied. Several changes in the sub-cellular levels have been noticed in these cells. These changes include occurrence of undigested aleurone grains, increase in number of mitochondria, dilation and fragmentation of ER and invigoration of plasmalemma. The ultrastructural changes are correlated with the disturbances in metabolic functioning of aleurone cells following NaCl salinity.

NaCl is known to cause marked and rapid changes in the metabolic activities of plant.2,3 Plants are more sensitive to salinity during germination and early seedling growth.3 Rice is one of the oldest and most important cereals. A considerable reduction in the percentage germination, dry matter accumulation and grain yield is reported in rice when subjected to NaCl.5 Even though many attempts have been made to understand the effect of salinity on different aspects of rice germination,2,3,5 little attention is paid on the structure of aleurone cells. Aleurone cells have an important role in the release of hydrolytic enzymes during germination. The present study was carried out with an aim to understand the structure of rice aleurone cells under the influence of NaCl.

Materials and Methods

Certified seeds of Oryza sativa L. var GR.11 (salt sensitive) were obtained from Agriculture Department, Gujarat, India. The seeds were pre-soaked in distilled water for 2 hr and dehusked without damaging the embryo and were surface sterilised with 0.1% HgCl₂ for 2 mm followed by thorough washing in distilled water. The seeds were blotted and a set of 25 seeds were transferred to sterile petriplates containing Whatman No. 1 filter paper moistened with 5 ml of—(a) sterile distilled water for control; and (b) 0.15 M, NaCl solution (which inhibited seedling growth and germination by 50%).

The seeds were allowed to germinate at 30±2°C in darkness for 120 hr. The embryo alongwith scutellum was separated and the region of grain just above scutellum was cut. Transverse sections of seeds measuring 2 to 3 mm thickness were processed for electron microscopy by fixation in 2.5%, glutaraldehyde and 1%, OsO₄. They were dehydrated in acetone and embedded in araldite 9. The ultra thin sections of seeds were stained with 2% uranyl acetate and lead citrate. They were observed under Philips CM 10 TEM at 80 KV.

Results

Control—The aleurone layer completely surrounded the grain and was tightly bound to the endosperm and scutellum. The cytoplasm of aleurone cells had many small vacuoles and a few aleurone grains at various stages of digestion (Fig. 1A, B, C). The diameter of grain varied from 2.2 to 4.5 μm. A number of spherosomes measuring 0.2 to 0.4 μm in diameter were found arranged in a distinct pattern around the periphery of the partially digested grain (Fig. 1B). They were more concentrated towards the plasma membrane (Fig. 1C). As the aleurone grains, spherosomes alongwith the vacuoles occupied much of the space in the aleurone cell, the cytoplasmic organelles appeared scanty. However, mitochondria, plastids, microbodies and free ribosomes were observed in the cytoplasm (Fig 1D, E). Occasionally rough ER was observed in the peripheral cytoplasm of the cells (Fig. 1E). The nucleus was conspicuous with a single nucleolus and dense nucleoplasm (Fig 1F).

NaCl treated—the aleurone cells of the seeds, germinated in NaCl solution for 120 hr, had dense cytoplasm with many small vacuoles (Fig 2A). The cytoplasmic organelles were in abundance. Aleurone grains were more in number and they were either in
Fig. 1—(A) Alerone cell of control rice seed (after 120 hr of germination); (B) Partially digested alerone grain surrounded by spherosomes; (C, D, E) Cytoplasm of alerone showing alerone grain (ag), mitochondria (m), ribosomes (r), rough endoplasmic endoplasmic reticulum (rer), mitochondria (mb); and (F) Alerone cell nucleus (n) with nucleolus (nu), plastid (pl)
undigested form or showing just the beginning of digestion (Fig. 2B, C). The globoid cavities (gc) of the aleurone grains were partially filled with phytin globoids (pg); (Fig 2B, C). Usually, the aleurone grain contained one globoid, but occasionally two (Fig.-2B, C). Their size varied from 0.5 to 3.0 \text{m in diameter}. The aleurone grains also contained granular membrane bound protein carbohydrate bodies (Fig. 2C). A well defined nucleus was seen in the center of aleurone cell. The chromatin in the nucleus appeared condensed compared to that of control (Fig. 3A).

Conspicuous changes were seen in distribution of cytoplasmic organelles such as mitochondria and ER. Mitochondria with indistinct cristae often appeared in large number in the cytoplasm (Fig. 3B). Besides, some of the mitochondria were also observed with vesiculated cristae (Fig. 3C). ER was cisterned and frequently dilated (Fig. 2D). Few ribosomes were seen free in the cytoplasm and also attached to ER (Fig. 3A). Plasmalemma formed vesicular invagination into cytoplasm. Vacuoles were often noticed with intravacuolar membranes in the cytoplasm of aleurone cells (Fig. 3D, E).

Discussion

Water uptake during germination process brings about changes in the cytoplasm and its organelles. The ultrastructural changes of membranes and organelles such as ER, mitochondria, ribosomes and microbodies during germination has been extensively studied and correlated to their metabolic activities in different plant systems\textsuperscript{12-15}.

Aleurone grains are the major sites of protein storage in aleurone cells. The vacuolation in these

![Figure 2](image-url)
Fig. 3—Aleurone cell of NaCl exposed rice seed showing—(A) Nucleus (n) with nucleous (nu) and condensed chromatin; (B) Group of mitochondria (m); (C) Mitochondria (m) with vesiculated cristae; (D, E) Invagination of membrane (arrow) into the vacuoles (v).

cells reflects mobilization of proteins for the growing embryo. Presence of numerous undigested aleurone grains indicated that NaCl inhibited the synthesis of proteolytic enzymes.

Structural changes in the aleurone cells of NaCl treated rice seeds clearly indicate their disturbed physiological functioning. Petruzzelli et al. suggested that the high density mitochondria observed in NaCl exposed cells may be a compensation for the damage in mitochondrial function. Vacuolation in mitochondria under stress is also reported by Smith et al. According to them vacuolation could be due to high accumulation of Na⁺ and/or Cl⁻ differences in ion compartmentalisation. Dictyosomes are reported to be active during germination. The failure to find them in the rice aleurone cells may be that they are few in number. The reduced occurrence of ribosomes could be due to water stress.

Salinity is known to cause changes in the membrane lipids and selective permeability of membranes. Invaginations of plasmalemma into the vacuoles to form endocytic vesicles may be a means to reduce NaCl toxicity. The chromatin condenses in the nuclei due to NaCl has been also reported in the root cells of barley and wheat embryos. According to them the chromatin condensation is due to suppression of nucleic acid biosynthesis in NaCl exposed cells. Thus it is concluded from the present observation that NaCl
inhibits seedling growth of rice by altering the cell structure and its metabolic functioning.

References
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