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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation　Studies on the halophilism in a halophyte, the common ice plant, *Mesembryanthemum crystallinum* L.
(塩生植物アイスプラントの好塩性に関する研究)

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Salinity is a serious environmental problem that reduces agricultural productivity. Improvement of salt tolerance of crops and application of salt tolerant plants as alternative crops are required to maintain stable and sustainable agriculture production. Halophytes are salt-tolerant plants that have ability to complete their life cycle under high salinity. Some of halophytes also require salt at some extents for their maximum growth, which is important trait for the salt adaptation of halophyte and referred to as halophilism. To elucidate the mechanisms of halophilism, the effects of NaCl on factors related to cell growth such as cell elongation, cell division, and ATP synthesis were examined on a halophyte, the common ice plant (*Mesembryanthenum crystallinum* L.).

1. Expression of ion homeostasis related genes associated with salt-stimulated cell elongation.

The salt-stimulated cell elongation and accumulation of ions K^+, Na^+, NO_3^- and Cl^- in the suspension cultured *M. crystallinum* cells grown under 100 mM NaCl condition have been observed in a previous study in our laboratory, suggesting that the cells may regulate ion homeostasis and osmotic adjustment for enhancing the cell elongation. In the present study, the factors that are induced by NaCl to promote the cell elongation and the mechanism of salt-stimulated cell elongation were elucidated. The growth of cells in culture medium contained 100 mM NaCl was higher than that in the medium contained PEG with the equivalent osmotic pressure, suggesting that the growth enhancement was due to the ionic effect more than osmotic effect of NaCl. Genes for Cl^- homeostasis, which encode a plasma membrane cation/Cl^- cotransporter (*McCCC1*) and a tonoplast Cl^-/H^+ antiporter (*McCLC1*), were annotated using the cDNA database of the ice plant. In addition, the expression analysis of genes encoding plasma membrane transporters and channels for incorporation of Na^+ (*McHKT1*), Cl^- (*McCCC1*), NO_3^- (*McNRT1*), K^+ (*McHAK1, McKmt1*), and water (*McMipC*); tonoplast transporters for vacuolar sequestration of Cl^- (*McCLC1*), Na^+ (*McNHX1*), and V-ATPase subunit c (*McVmac1*); and enzymes for synthesis of proline (*McP5CS*), ononitol (*McImt1*), and cell wall metabolism xyloglucan endotransglucosylase/hydrolase (*McXTH*) in the cells treated with 100 mM NaCl showed that the expression of *McHKT1*, *McCCC1*, *McCLC1*, *McNHX1*, *McVmac1*, *McKmt1*, *McNRT1*, *McP5CS*, and *McImt1* were higher in the salt treated cells than that in the untreated cells. The results suggested that these genes were involved in the accumulation of ions and compatible solutes, which might contribute to the enhancement of turgor pressure and cell elongation in the ice plant cells grown positively with NaCl.

2. Expression of cell cycle related genes associated with salt-stimulated cell division

The previous study also showed that the enhancement of cell division contributed to the halophilism of the ice plant. To elucidate the factors associated with the salt-stimulated cell division, in the present study, the G1-S phase synchronized suspension cells were produced by phosphate starvation for 72 h, and the expression of cell cycle related genes was analyzed on the synchronized cells grown positively with 25 mM NaCl. In addition, fifteen cell cycle related genes that are associated with the regulation of progression of G1 phase (*McCycD2;1, McKRP2/ICK2, McCDKA;1,* and *McCycD3;1*), S phase (*McHistone H4, McKRP3, McCKS1At, McE2Fb,* and *McCDKA;1*), G2 phase (*McCDKB1;1, McKRP4, McCycD1;1, McCycB2;1,* and *McCDKA;1*), and M phase (*McCDKB2;2, McCycA2;1, McCycB1;1, McCycD3;1, McCDKA;1,* and *McCKS1At*) were annotated using the cDNA database of the ice plant. Using the synchronized cells, the expression analysis of *McCycD2;1*, *McCycD3;1*, *McHistone H4*, and *McKRP3* showed that the expression of *McCycD2;1*, *McCycD3;1* were higher in the salt treated cells than that in the untreated cells, suggesting that NaCl might promote the progression of G1 phase that contributed to the salt-stimulation cell division.

3. Expression of mitochondrial ATP synthesis related genes associated with salt-stimulated ATP synthesis

The ATP synthesis is considered to increase to meet the increased energy demand of halophilism of the ice plant. In the present study, statistical analysis on the data of ATP synthesis obtained in the previous study, which measured the ATP synthesis rate in mitochondria isolated from the ice plants grown with 0, 100, 400 mM NaCl in the assay mixtures contained 50-350 mM NaCl with fixed 2 MPa osmotic pressure, showed that the ATP synthesis increased with increasing NaCl concentrations, up to 350 mM, in the assay mixtures. In addition, the ATP synthesis rate was higher in mitochondria isolated from the plant grown with NaCl than that of the plant grown without NaCl. These results suggested that NaCl stimulates the ATP synthesis, which contributes to the halophilism of the ice plant. To elucidate the factors involved in salt-stimulated ATP synthesis, nine genes encoding mitochondrial ATP synthesis related proteins such as a beta subunit of ATP synthase (*McATPF1b*); subunits of electron transport chain (ETC) complexes such as a 76 kDa subunit of complex I (*McCI76*), flavoprotein subunit of complex II (*McSDH1-1*), 6B subunit of complex III (*McCOX6B-1*), 7 subunit of complex IV (*McQCR7*) and alternative oxidase (*McAOX1a*); subunits of tricarboxylic acid (TCA) cycle related enzymes such as an E1 alpha subunit of pyruvate dehydrogenase (*McPDHE1α*) and malate dehydrogenase (*McmMDH1*); and mitochondrial phosphate transporter (*McMPT1*) were annotated using the cDNA database of the ice plant. The expression of these genes and adenylate transporter *McANT2* were analyzed on the suspension cultured cells grown positively with 100 mM NaCl. The expression of *McATPF1b*, *McCOX6B-1*, *McCI76*, *McQCR7*, *McSDH1-1*, *McmMDH1*, *McPDHE1α*, and *McANT2* was higher, but the expression of *McAOX1a* was lower in the salt treated cells than that in the untreated cells. These results suggested that *McATPF1b*, *McCOX6B-1*, *McCI76*, *McQCR7*, *McSDH1-1*, *McmMDH1*, *McPDHE1α*, *McANT2*, *McAOX1a* were involved in the salt-stimulated ATP synthesis. It also suggested that the ATP synthase, ETC, and TCA might be promoted by salt to enhance the ATP synthesis.