NaCl-stimulated expression of genes for ion homeostasis and cell cycle regulation related to the halophilism in a halophyte, *Mesembryanthemum crystallinum* L.

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Introduction: A halophyte, the common ice plant (*Mesembryanthemum crystallinum* L.) shows the maximal growth under salinity, in which almost all crops would die. The promotion growth by salt, which is referred to as halophilism, is an important trait for adaptation to salinity, but the mechanism is still unclear. Growth of plants is determined by cell division and cell elongation; thus, factors involved in these two physiological processes of the growth would contribute to the halophilism. To elucidate factors involved in the halophilism, in the present study we tested effects of NaCl on the growth enhancement and expression of genes responsible for the ion homeostasis, cell wall elasticity, and cell cycle regulation.

Materials and Methods: The suspension-cultured cells were produced according to procedures established previously in our laboratory (Konishi 2016), in which the cells showed the growth enhancement with 25-100 mM NaCl. The cells were also cultured in the medium containing polyethylene glycol (PEG) and NaCl at equivalent osmotic pressures to test the effects of osmotic pressure and Na^+ and Cl^- on the growth enhancement. A procedure of cell cycle synchronization using the phosphate starvation was established to produce the synchronous cells during the shift of G1 to S phase of the cell cycle for analyzing the expression of cell cycle-related genes. Transcript abundance of the genes in the cells untreated and treated with 25 mM NaCl (for the analysis of cell cycle-related genes) and 100 mM NaCl (for the analysis of ion homeostasis-related genes) were analyzed using semi-quantitative or quantitative RT-PCR.

Results and Discussions: The growth of cells was increased with NaCl and PEG, but the enhancement of growth was higher in the medium with NaCl than that with PEG, suggesting that the growth was promoted by the ionic effect rather than the osmotic effect of NaCl. The expression analysis of genes encoding plasma membrane transporters and channels for incorporation of Na^+ (*McHKT1*), K^+ (*McKmt1* and *McHAK1*), Cl^- (*McCCC1*), NO_3^- (*McNRT1*), and water (*McMipC*); tonoplast antiporters of H^+/Cl^- (*McCLC1*) and Na^+/H^+ (*McNHX1*) and a V-ATPase subunit c (*McVmac1*) for the vacuolar sequestration of Na^+ and Cl^-, and enzymes catalyzing synthesis of proline (*McP5CS*) and ononitol (*McImt1*) and cell wall metabolism (*McXTH*) showed that the expression of *McHKT1*, *McKmt1*, *McCCC1*, *McNRT1*, *McCLC1*, *McNHX1*, *McVmac1*, *McP5CS*, and *McImt1* were higher in the salt-treated cells than that in the untreated cells. Also, the expression analysis of cell cycle-related genes responsible for regulation of G1 and S phase progression such as G1 cyclins (*McCycD2;1* and *McCycD3;1*) and S phase regulators (*McHistone H4* and a cyclin-dependent kinase inhibitor *McKRP3*) in the synchronous cells showed that the expression of *McCycD2;1* and *McCycD3;1* were higher in the salt-treated cells. The increased expression of *McHKT1*, *McKmt1*, *McCCC1*, *McNRT1*, *McCLC1*, *McNHX1*, *McVmac1*, *McP5CS*, *McImt1*, *McCycD2;1*, and *McCycD3;1* suggest that these genes are involved in the halophilism of the ice plant, as factors which might contribute to the ion homeostasis, osmotic adjustment, and cell cycle regulation for the growth enhancement.

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