Summary of Doctoral Thesis

Enrollment year: 2015 y 10 m
UGAS Specialty: Cryobiosystems
Name ASHRAF MOHAMMAD ARIF

Title	The role of membrane proteins in abiotic stresses in *Arabidopsis thaliana*

Summary

The growth and development of plants are constantly challenged due to the change in the environment or stressful conditions. These unfavorable environmental conditions include biotic stress, such as invasion of virus, parasites, and insects, and abiotic stress, such as drought, heat, cold, salinity, nutrient deficiency, and excess of toxic compounds like arsenate, cadmium, cesium in the soil. Among these, temperature stress has become the major concern as heat and cold stress lead to other abiotic stresses. For instance, heat and cold stress cause drought and osmotic stress, respectively. Between high and low temperature stress, the decrease in temperature and the increased length of winter have become the major limiting factor to provide the favorable growth temperature for most of the crop plants (Chapter 1).

Membrane proteins play major role in the cold stress response. Because, low temperature results in membrane rigidification. The rigidification affects membrane residing proteins including transporters, receptors and receptor kinases. Previous study corroborates with this hypothesis as auxin efflux carrier PIN2 (PIN-FORMED2) is targeted by cold stress. PIN2, polar localized transporter, is continuously endocytosed inside the cell from plasma membrane (PM) through endocytosis and phosphorylated inside the cytoplasm for its activation. Cold stress specifically disrupts this PM – cytosol – PM communication cycle of PIN2 (Chapter 1 and 2).

In general, newly synthesized proteins from rough endoplasmic reticulum (RER) move to trans-Golgi network (TGN), subsequently secrete from TGN to form vesicles and reach destination organelles. Furthermore, inclusion and exclusion of proteins in cell involve the movement from PM (endocytosis) and to PM (exocytosis), respectively. These dynamic movements are known as protein trafficking. Both high and low temperature interrupt the protein trafficking and as a result, protein dynamics is considered as major target for temperature stress. Unfortunately, in depth mechanism of temperature-mediated inhibition of protein trafficking is still elusive (Chapter 1).

In this research, I have used known protein trafficking mutants and identified GNOM,

Guanine nucleotide Exchange Factors for ADP Ribosylation Factor (ARF-GEF), as one of the cold stress response regulators. GNOM is localized in the both cytosol and PM. Weak mutant of GNOM, *gnom*$^{B/E}$, is hypersensitive to cold and GNOM overexpressing engineered line (GNOM^{M696L}) is resistant to cold from root phenotype and whole plant level. GNOM-engineered line contains single point mutation in the regulatory SEC7 domain, responsible for membrane associated GTPase activity, and this point mutation induces the overexpression of GNOM. GNOM participates in the recycling endosomal pathway and helps PIN2 trafficking between PM and cytosol. Under cold stress, the endocytosis of PIN2 is almost disappeared, but in the GNOM-engineered line, the PIN2 endocytosis persists even after cold treatment. Altogether, it suggests that the persistent PIN2 endocytosis and maintenance of proper auxin homeostasis under cold stress are mediated through the overexpression of GNOM (Chapter 2).

Metal contamination is another important abiotic stress. There are approximately 90 naturally occurring elements and a portion of them are available in the soil due to their solubility. Among them, K, P, Fe, Mo, and Mn are important as micronutrients, whereas Zn, Ni, Cu are trace elements but toxic for plants at higher concentrations. But the other elements such as As, Cs, Cd, Hg, and Pd have no biological functions and toxic for plants. These components are easily incorporated into the plants and eventually become part of our food cycle. Metal toxicity causes inhibition of growth, cell death, DNA damage, protein oxidation, alteration of water potential, ROS production, reduction in photosynthesis, and other detrimental effects on plants. As a consumer of those contaminated products, the human and animal health are prone to physiological abnormalities.

In these recent years, cesium drew our attention most due to the nuclear power plant accident in Fukushiam, Japan during the Tsunami of 2011. Surrounding area was declared unsuitable for crop production due to contaminated radio cesium. Due to its chemical similarity with potassium, plants easily taken up cesium using potassium transporters. In such a circumstance, phytoremediation is of the approaches to overcome this issue. For this purpose, understanding the cesium transport system is the first step. Unfortunately, so far identified cesium transporters are mostly known potassium transporters. Manipulation of potassium transporters will alter the indispensable micronutrients for plants. Additionally, plants uptake cesium from soil in the presence of low potassium concentration, which is also a rare event. Taken together, alteration of potassium transporters is irrational to get rid of cesium toxicity (Chapter 1).

In the search for alternative cesium transporters, gene expression of cesium intoxicated plants compared with potassium starved plants suggests that a group of ABC transporters are differentially expressed. ABC transporters family is the largest group of transporters in both animal and plant cells. Moreover, ABC transporters are involved in detoxification of animal cells. At the same time, several plant ABC transporters have been reported as metal transporters. Hence, I focused on the screening of ABC transporters to find alternative cesium transporter (Chapter 1).

Based on the screening of ABC transporters, I have identified two ABC transporters, ABCG33 and ABCG37, as cesium influx carriers, which function redundantly. The gain-of-function mutant of ABCG37, *abcg37-1*, is hypersensitive to cesium, although single knockout of ABCG37 (*abcg37-2*, *abcg37-3*, *abcg37-4*) and ABCG33 (*abcg33-1*) have wild-type like phenotype in the presence of cesium. Double knockout mutant (*abcg37-2 abcg33-1*) shows the resistant phenotype and reduce uptake of cesium. Combining the knowledge of these transporters with existing low cesium containing crop plants will help to develop varieties capable of growing in cesium contaminated soil (Chapter 3).

This thesis work was focused on the understanding of mechanism of two major abiotic stress (cold and cesium stress). Interestingly, during this process, I have found that membrane proteins act as convergence point for both abiotic stresses. During cold stress, the membrane localized GNOM function as endosomal trafficking components and response regulators of low temperature. And, plasma membrane localized ABCG37 and ABCG33 work as cesium influx carriers. Taken together, this research work conclude the role of membrane proteins as a major regulator of abiotic stresses (Chapter 2, 3 and 4).