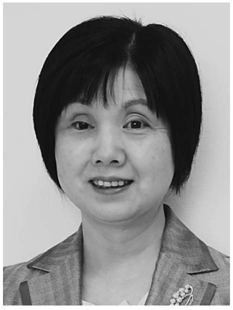
Japan Academy Prize to:

Kazuo Shinozaki
Senior Visiting Scientist,
RIKEN Center for Sustainable Resource Science
Distinguished Professor,
Nagoya University
RIKEN Honorary Science Advisor

and

Kazuko Yamaguchi-Shinozaki
Professor,
Tokyo NODAI Research Institute,
Tokyo University of Agriculture
Emeritus Professor,
The University of Tokyo



for "The Regulatory Network Involved in Environmental Stress Responses and Tolerance in Plants" (Joint Research)

Outline of the work:

Plants need to develop tolerance to environmental stresses such as drought, extreme temperatures, and high salt content for ensuring their survival under such conditions. Dr. Kazuo Shinozaki and Dr. Kazuko Yamaguchi-Shinozaki through their collaborative research have conducted systematic pioneering studies on the identification and regulation of genes that are related to environmental stress response and acquisition of resistance in plants, making great achievements in this field of research.

Initially, they searched for genes that are induced by drought and low temperature and observed that the expression of several genes is induced at the transcriptional level in response to these stresses. Then, through detailed analysis of the transcriptional patterns of genes that respond to drought stress, they discovered that in addition to the known abscisic acid (ABA)-dependent pathway, there exists an ABA-independent pathway, which is involved in abiotic stress responses as well. Furthermore, they succeeded in identifying not only the regulatory promoter regions of these genes but also the transcription factors that bind to them. These are necessary for the expression of environmental stress-inducible genes. It is worthy of note that the novel ABA-independent *cis*-acting region, DRE (Dehydration Responsive Element), and the transcription factor DREB (DRE Binding protein) family identified by them have been mentioned in various plant science textbooks.

Dr. Shinozaki's collaborative team has also achieved many positive results in understanding the control system of ABA. They identified AREB (ABA Responsive Element Binding protein), which binds to the *cis*-

element ABRE (ABA Responsive Element) of the concerned gene, as a transcription factor involved in ABA-dependent transcriptional regulation, and also observed that AREB is activated through phosphorylation. Furthermore, they identified major enzymes involved in ABA biosynthesis and degradation, and also discovered the transporter ABCG25, which is involved in transport between tissues; thus, elucidating the whole picture of the ABA-dependent control of responses to drought stress.

Drs. Shinozaki and Yamaguchi-Shinozaki also identified important regulatory factors involved in the perception of environmental stress and the transmission of related signals. In particular, they focused on the phosphorylation status of enzymes involved in signal transduction, demonstrating that MAP kinases and SnRK2 kinases play an important role in intracellular signal transduction related to drought stress-induced ABA response. Furthermore, they made significant contributions to the field of research on intracellular signal transduction related to environmental stress responses, including the identification of factors upstream of SnRK2 and the discovery of histidine kinase, which is a receptor for osmotic stress.

Signals about water deficit are transmitted from the roots to the leaves via the vascular system of the stems, and transpiration is suppressed by inducing the closure of the stomata. Drs. Shinozaki and Yamaguchi-Shinozaki discovered a CLAVATA3/ EMBRYO-SURROUNDING REGION-RELATED 25 (CLE25) peptide that is involved in this process. CLE25 is synthesized in the roots in response to water stress, transported from the roots to the leaves through the xylem vessels, and binds to the membrane receptors of the leaf cells to induce ABA synthesis. As a result, the stomata are closed and the loss of water through transpiration is suppressed. This study is notable for being the first to demonstrate that peptides are involved in long-distance signal transduction from the roots to the leaves in response to drought stress.

Drs. Shinozaki and Yamaguchi-Shinozaki have also contributed to applied research to develop crops resistant to environmental stresses. Through international collaborations, they have developed drought-stress-tolerant and salt-stress-tolerant rice, wheat, and soybean. Many achievements have also been made regarding their applicability in the field. In particular, in joint research with the Institute of the Consultative Group on International Agricultural Research (CGIAR), they have succeeded in strengthening drought tolerance in rice and soybeans in arid regions and increasing their yield.

The publications based on the results obtained by the joint research conducted by Dr. Kazuo Shinozaki and Dr. Kazuko Yamaguchi-Shinozaki have received a particularly high number of citations. Both have also been recognized as Highly Cited Researchers in the field of plant sciences by Thomson Reuters/Clarivate Analytics continuously since 2014. The two have also jointly received the Japanese Society of Plant Physiology Prize and the Tsukuba Prize. Dr. Shinozaki has received the Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science, and Technology, the Medal with Purple Ribbon; the Person of Cultural Merit Award; the International Prize for Biology; the Khalifa International Award, and is elected as an international member of the National Academy of Science (USA) and as a Pioneer Member of the American Society of Plant Biologists. Dr. Yamaguchi-Shinozaki has also received the Tokyo Techno Forum 21 Gold Medal Award; the Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science, and Technology, and the MIDORI Academic Award. Hence it can be concluded that both Dr. Kazuo Shinozaki and Dr. Kazuko Yamaguchi-Shinozaki have made outstanding achievements in the field of plant biology and agricultural science.

List of Main Publications

1. Yamaguchi-Shinozaki K. and Shinozaki K. (1994) A novel *cis*-acting element in an Arabidopsis gene is involved in responsiveness to drought, low-temperature, or high-salt stress. *Plant Cell* 6, 251–264.
2. Mizoguchi T., Irie K., Hirayama T., Hayashida N., Yamaguchi-Shinozaki K., Matsumoto K., and

Shinozaki K. (1996) A gene encoding a mitogen-activated protein kinase kinase kinase is induced simultaneously with genes for a mitogen-activated protein kinase and an S6 ribosomal protein kinase by touch, cold, and water stress in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U.S.A.* 93, 765–769.

3. Liu Q., Kasuga M., Sakuma Y., Abe H., Miura S., Yamaguchi-Shinozaki K., and Shinozaki K. (1998) Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low- temperature-responsive gene expression, respectively, in Arabidopsis. *Plant Cell* 10, 1391–1406.
4. Kasuga M., Liu Q., Miura S., Yamaguchi-Shinozaki K., and Shinozaki K. (1999) Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nat. Biotechnol.* 17, 287–291.
5. Urao T., Yakubov B., Satoh R., Yamaguchi-Shinozaki K., Seki M., Hirayama T., and Shinozaki K. (1999) A transmembrane hybrid-type histidine kinase in Arabidopsis functions as an osmosensor. *Plant Cell* 11, 743–754.
6. Uno Y., Furihata T., Abe H., Yoshida R., Shinozaki K., and Yamaguchi-Shinozaki K. (2000) *Arabidopsis* basic leucine zipper transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high-salinity conditions. *Proc. Natl. Acad. Sci. U.S.A.* 97, 11632–11637.
7. Seki M., Narusaka M., Kamiya A., Ishida J., Satou M., Sakurai T., Nakajima M., Enju A., Akiyama K., Oono Y., Muramatsu M., Hayashizaki Y., Kawai J., Carninci P., Itoh M., Ishii Y., Arakawa T., Shibata K., Shinagawa A., and Shinozaki K. (2002) Functional annotation of a full-length *Arabidopsis* cDNA collection. *Science* 296, 141–145.
8. Yoshida R., Hobo T., Ichimura K., Mizoguchi T., Takahashi F., Aronso J., Ecker J.R., and Shinozaki K. (2002) ABA-activated SnRK2 protein kinase is required for dehydration stress signaling in *Arabidopsis*. *Plant and Cell Physiol.* 43, 1473–1483.
9. Abe H., Urao T., Ito T., Seki M., Shinozaki K., and Yamaguchi-Shinozaki K. (2003) Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. *Plant Cell* 15, 63–78.
10. Furihata T., Maruyama K., Fujita Y., Umezawa T., Yoshida R., Shinozaki K. and Yamaguchi-Shinozaki K. (2006) Abscisic acid-dependent multisite phosphorylation regulates the activity of a transcription activator AREB1. *Proc. Natl. Acad. Sci. U.S.A.* 103, 1988–1993.
11. Sakuma Y., Maruyama K., Qin F., Osakabe Y., Shinozaki K. and Yamaguchi-Shinozaki K. (2006) Dual function of an *Arabidopsis* transcription factor DREB2A in water-stress- and heat-stress-responsive gene expression. *Proc. Natl. Acad, Sci. U.S.A*. 103, 18828–18833.
12. Tran L.-S. P., Urao T., Qin F., Maruyama K., Kakimoto T., Shinozaki K., and Yamaguci-Shinozaki K. (2007) Functional analysis of AHK1/ATHK1 and cytokinin receptor histidine kinases in response to abscisic acid, drought and salt stresses in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* 104, 20623–20628.
13. Umezawa T., Sugiyama N., Mizoguchi M., Hayashi S., Myouga F., Yamaguchi-Shinozaki K., Ishihama Y., Hirayama T., and Shinozaki K. (2009) Type 2C protein phosphatases directly regulate abscisic acid-activated protein kinases in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* 106, 17588–17593.
14. Kuromori T, Miyaji T, Yabuuchi H, Shimizu H, Sugimoto E, Kamiya A, Moriyama Y., and Shinozaki K. (2010) ABC transporter AtABCG25 is involved in abscisic acid transport and responses. *Proc. Natl. Acad. Sci. U.S.A.* 107, 2361–2366.
15. Takahashi F., Mizoguchi T., Yoshida R., Ichimura K., and Shinozaki K. (2011) Calmodulin-dependent activation of MAP kinase for ROS homeostasis in *Arabidopsis*. *Mol. Cell* 41, 649–660.
16. Umezawa T., Sugiyama N., Takahashi F., Anderson J.C., Ishihama Y., Peck S.C., and Shinozaki K. (2013) Genetics and phosphoproteomics reveal a protein phosphorylation network in the abscisic acid signaling

pathway in *Arabidopsis thaliana*. *Sci. Signal.* 6, rs8.

17. Morimoto K., Ohama N., Kidokoro S., Mizoi J., Takahashi F., Todaka D., Mogami J., Sato H., Qin F., Kim J.-S., Fukao Y., Fujiwara M., Kazuo Shinozaki S., and Yamaguchi-Shinozaki K. (2017) BPM-CUL3 E3 ligase modulates thermotolerance by facilitating negative regulatory domain-mediated degradation of DREB2A in *Arabidopsis, Proc. Natl. Acad. Sci. U.S.A.* 114, E8528–E8536.
18. Soma F., Mogami J., Takuya Yoshida T., Abekura M., Takahashi F., Satoshi Kidokoro S., Mizoi J., Shinozaki K., and Yamaguchi-Shinozaki K. (2017) ABA-unresponsive SnRK2 protein kinases regulate mRNA decay under osmotic stress in plants, *Nat. Plants* 3, 16204.
19. Selvaraj M.G., Ishizaki T., Valencia M., Ogawa S., Dedicova B., Ogata T., Yoshikawa K., Maruyama M., Kusano M., Saito K., Takahashi F., Shinozaki K., Nakashima K., and Ishitani M. (2017) Overexpression of an *Arabidopsis thaliana* galactinol synthase gene improves drought tolerance in transgenic rice and increased grain yield in the field. *Plant Biotechnol. J.* 15, 1465–1477.
20. Kidokoro S., Yoneda K., Takasaki H., Takahashi F., Shinozaki K., and Yamaguchi-Shinozaki K. (2017) Different cold-signaling pathways function in responses to rapid and gradual decreases in temperature. *Plant Cell* 29, 760–774.
21. Takahashi F., Suzuki T., Osakabe Y., Betsuyaku S., Kondo Y., Dohmae N., Fukuda H., Yamaguchi-Shinozaki K., and Shinozaki K. (2018) A small peptide modulates stomatal control via abscisic acid in long-distance signaling. *Nature* 556, 235–238.
22. Soma F., Takahashi F., Suzuki T., Shinozaki K. and Yamaguchi-Shinozaki K. (2020) Plant Raf-like kinases regulate the mRNA population upstream of ABA-unresponsive SnRK2 kinases under drought stress. *Nat. Commun.* 11, 1373.
23. Kidokoro S., Hayashi K., Haraguchi H., Ishikawa T., Soma F., Konoura I., Toda S., Mizoi M., Suzuki T., Shinozaki K., and Yamaguchi-Shinozaki K. (2021) Posttranslational regulation of multiple clock-related transcription factors triggers cold-inducible gene expression in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A*. 118, e2021048118.
24. Kim J.S., Sakamoto Y., Takahashi F., Shibata M., Urano K., Matsunaga S., Yamaguchi-Shinozaki K. and Shinozaki K. (2022) *Arabidopsis* TBP-ASSOCIATED FACTOR 12 ortholog NOBIRO6 controls root elongation with unfolded protein response cofactor activity. *Proc. Natl. Acad. Sci. U.S.A.* 119, e2120219119.