Chapter 1-1

Development of biotechnologies and biotech crops for stable food production under adverse environments and changing climate conditions

Kazuo Nakashima [1*]

1 Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan

** Corresponding author; E-mail: kazuo.nakashima@affrc.go.jp*

Abstract

Developing regions, such as sub-Saharan Africa, are rapidly growing in population. In sub-Saharan Africa, 215 million people are currently malnourished. In these areas, the poor environmental conditions for agricultural production and the vulnerability to climate change make agricultural productivity low. Hence, there is a need to develop crops with improved tolerance to adverse environments and climate change. To ensure food and nutritional security, we have been working on improving crops such as rice and soybean using marker-assisted selection and biotechnology. Biotechnology, especially genetic modification, is being used to develop crops that have increased tolerance to adverse environmental conditions such as drought. We have promoted international collaborative research projects to enhance the drought tolerance of globally important crops such as rice, wheat, and soybean. Throughout the project, we have shown that overexpression of genes encoding key stress-related transcription factors and enzymes improves drought tolerance in transgenic crops such as rice and soybean. We hope that biotechnology-based agricultural research efforts and biotechnology crops can contribute to the security of food and nutrition in developing regions and globally.

1. Introduction

Food insecurity is widespread in Africa and Asia. Currently, 215 million people are malnourished in sub-Saharan Africa. Goal 2 of the United Nations' 17 Sustainable Development Goals (SDGs) aims to end hunger, achieve food security and improved nutrition, and promote sustainable agriculture (United Nations Information Centre 2018). The full potential of agriculture in developing regions, including sub-Saharan Africa, has not been realized due to adverse environmental and climatic conditions that impose environmental stress (abiotic stress) such as reduced soil fertility, and drought, and biological stress such as pests, and diseases. Haefele et al. (2014) assessed soil quality in all rice producing areas around the globe. Globally, one-third of the total rice is grown on very poor soils. Rice production in good soils is largest in Asia (47%)

while it is less common in the Americas (28%) and accounts for only 18% in Africa. They report that the most common problems pertaining to soil chemistry in rice fields are: very low inherent nutrient status (35.8 million ha), very low pH (27.1 million ha), and high P fixation (8.1 million ha); widespread physical problems of the soil, especially severe in rainfed environments are: very shallow soils and low water-holding capacity. In recent years, droughts have frequently occurred around the world, causing serious damage to agricultural production. The National Agriculture and Food Research Organization (NARO), Japan has published the geographical distribution of grain production damage in the world due to drought (Kim et al. 2019). Analysis of precipitation and grain yield data of the last 27 years (1983-2009) showed that three quarters (450 million ha) of the world's major crop (corn, rice, soybean, wheat) cultivation area had been damaged by drought, and the total grain production damage estimated from this analysis and the country's producer price (2005) was about $166 billion. In the past 27 years (1983-2009), the cultivation area of cereals that has been damaged by at least one drought is 161 million ha (75% of the world's harvested area). About 124 million ha of corn (82%), 102 million ha of rice (62%), and 67 million ha of soybeans (91%) have been damaged. The average yield loss from a single drought in 27 years was 8% for wheat (0.29 tons per hectare), 7% for corn (0.24 tons), 3% for rice (0.13 tons), and 7% for soybean (0.15 tons).

The Stable Agricultural Production Program in Japan International Research Center for Agricultural Sciences (JIRCAS) aims to improve agricultural productivity, stability, and nutrition in developing countries by utilizing stable production technology for agricultural products in adverse environments such as the tropics. We have developed technologies and crops that are highly productive and adaptable to changing adverse environmental and climatic conditions to ensure food and nutrition security in developing regions such as sub-Saharan Africa. To date, we have developed crops such as rice and soybeans that have improved resistance to adverse environmental conditions, such as high temperature, high salt, and diseases, by using marker-assisted selection (MAS). Biotechnology, especially genetically modified (GM) technology, is expected to develop GM or biotechnology crops that have increased tolerance to adverse environmental conditions, including drought. We promoted international collaborative research projects to develop drought-tolerant crops such as rice, wheat, and soybean. This chapter introduces an outline of the research on biotechnology and the development of biotechnology crops for stable food production under adverse conditions and climate change.

2. Development of biotechnology to improve drought tolerance in crops

The frequency and severity of drought in the world have increased in the recent years, and the resulting agricultural damage has been more severe than ever. Many small-scale farmers in the developing world use rain-fed cultivation for rice and other crops, however, it is affected more by drought than irrigation cultivation. Such rainfed areas are closely linked to poor areas. Thus, drought has a major impact on social issues in developing countries. We conducted research using biotechnology to identify candidate genes to improve the drought tolerance of crops. To identify these genes, we investigated the molecular mechanisms involved in the environmental stress response in rice and Arabidopsis (*Arabidopsis thaliana*) as model plants. The results showed that many factors in gene expression were induced and/or activated for the role of stress response and tolerance in controlling stress perception, signal transduction, and drought tolerance in plants (**Fig. 1**).

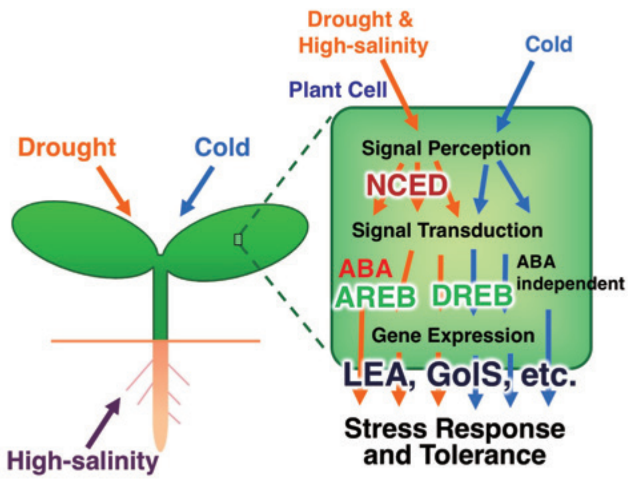

Fig. 1. Outline of the molecular network in environmental stress response and tolerance in plants

Abscisic acid (ABA) is an important plant hormone that regulates gene expression and physiological responses, including stomatal closure under environmental stresses such as drought. The *NCED3* gene encodes a key enzyme, 9-cis-epoxycarotenoid dioxygenase (NCED) 3 in the ABA biosynthetic pathway (**Fig. 1**), the expression of which is strongly induced by water-deficient stress in many kinds of plants, including Arabidopsis (Iuchi et al. 2001).

Stress-inducible transcription factors (TFs), such as the dehydration-response element (DRE)-binding protein (DREB) and the ABA-response element (ABRE)-binding factor (AREB), play important roles in regulating the stress response (**Fig. 1** to **3**; reviewed by Nakashima et al. 2014). Under environmental stress conditions, plants perceive a stress signal and transmit it to transcription factors such as DREB (**Fig. 2**). DREB TF is an important element that binds to the DRE *cis*-element of many kinds of environmental stress-responsive promoters and can switch on the expression (transcription) of stress-responsive genes. AREB can switch on gene expression in the ABA response. We showed that many types of transcription factors, including DREB and AREB, regulate different types of stress-inducible promoters through specific binding to *cis*-elements such as DRE and ABRE for plant stress response and tolerance (**Fig. 3**).

TFs regulate the expression of target genes encoding important metabolic proteins that protect cells from dehydration, including late embryogenesis abundant (LEA) proteins, proteases, chaperones, water channel proteins, and enzymes for the synthesis of osmoprotectants (compatible solutes) such as sugars and proline. The osmoprotectants include galactinol synthase (GolS; **Fig. 1**), which is the key enzyme in the production of raffinose family oligosaccharides (RFOs). RFOs are thought to influence drought tolerance by regulating osmotic potential and protecting enzymes and membranes during exposure to environmental stresses. *GolS* genes are upregulated by abiotic stresses in many kinds of plants such as Arabidopsis (Taji et al. 2002).

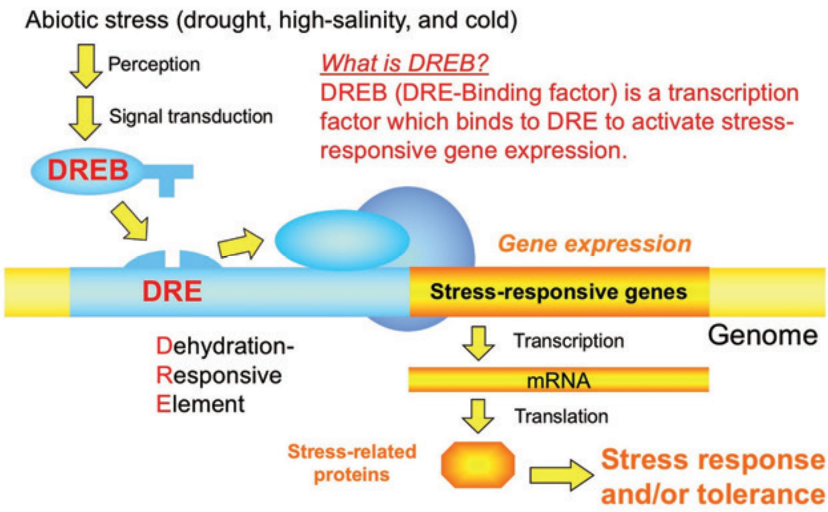

Fig. 2. Dehydration-response element-binding protein (DREB) plays an important role in regulating stress response and tolerance in plants

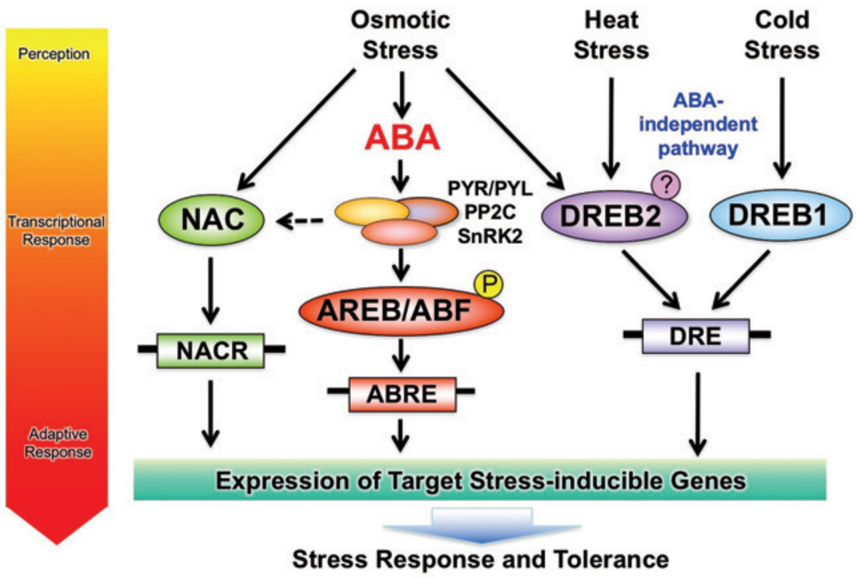

Fig. 3. Major transcriptional networks under environmental stress conditions revealed by our molecular biology studies in plants.

Environmental stresses such as osmotic stress, heat stress, and cold stress result in the expression and activation of transcription factors. The transcription factor binds to the specific *cis*-element in the promoter region of the target gene and induces the expression of the gene. Gene products function in stress response and tolerance. Ellipses indicate transcription factors. Boxes indicate *cis* elements. Details are provided in the text.

Overexpression of genes that are key to environmental stress response has been shown in greenhouse experiments to enhance stress tolerance in rice and Arabidopsis (reviewed in Nakashima et al. 2014). For example, overexpression of the *DREB1* gene increased the tolerance of Arabidopsis to drought, high salinity, and low temperature (**Fig. 4** to **6**; Liu et al. 1998; Kasuga et al. 1999). However, constitutive overexpression of *DREB1A* using the cauliflower mosaic virus *35S* promoter induced growth defects. A stress-responsive promoter such as *Arabidopsis RD29A* has been able to avoid delayed growth by *DREB1A* expression (Kasuga et al. 1999). We have worked with various research institutions to determine whether such genes can improve stress tolerance in other crops in the field (reviewed in Nakashima et al. 2014).

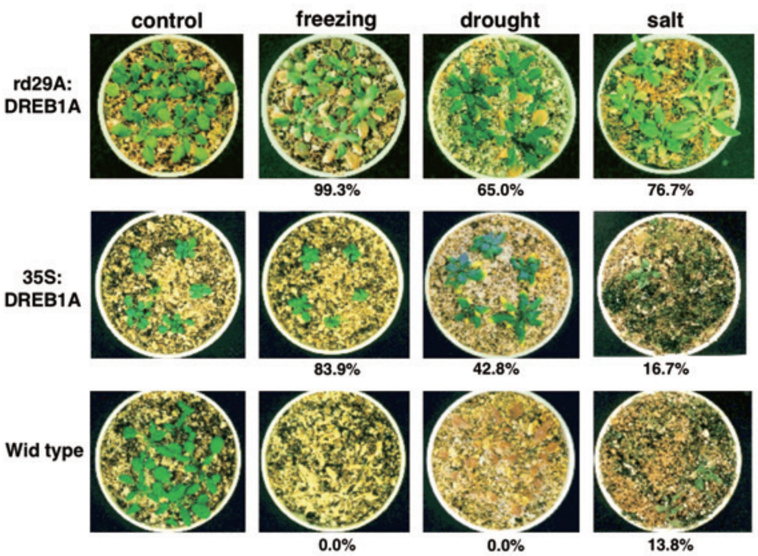

Fig. 4. Constitutive and stress-induced overexpression of *DREB1* in Arabidopsis improves tolerance to multiple stresses and alters plant growth.
Overexpression of the *DREB1* gene increased tolerance to drought, high salinity, and low temperature in Arabidopsis. However, constitutive overexpression of *DREB1A* using the cauliflower mosaic virus *35S* promoter induced growth defects. A stress-responsive promoter such as *Arabidopsis RD29A* allows the avoidance of growth defects caused by *DREB1A* expression. The figure was adapted from Kasuga et al. (1999).

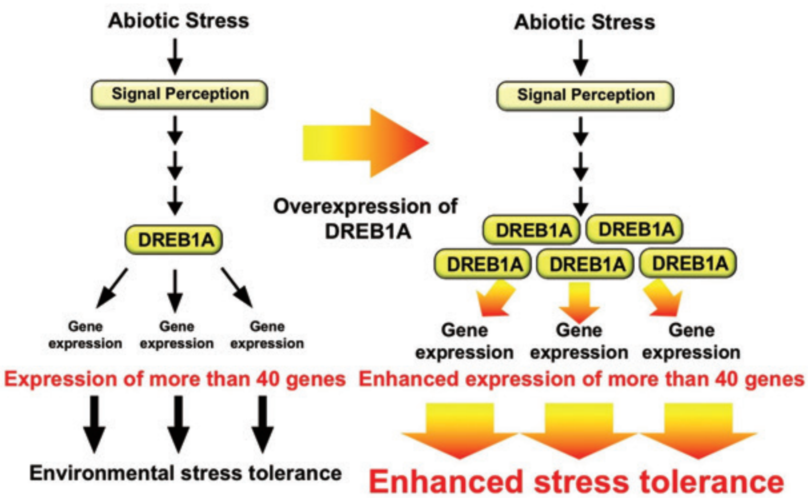

Fig. 5. Overexpression of DREB1A, a transcription factor that is key in controlling environmental stress in Arabidopsis, can enhance plant stress tolerance.

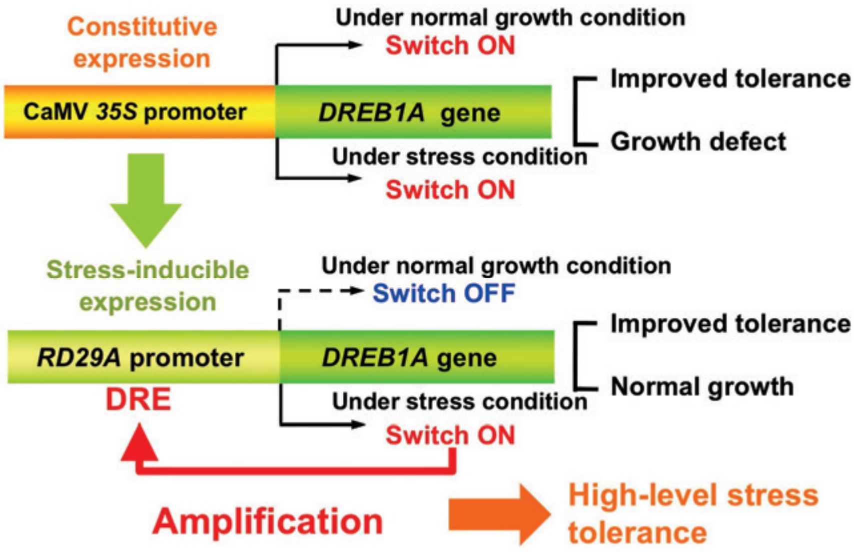

Fig. 6. *DREB1A* expression using a stress-responsive promoter, such as *Arabidopsis RD29A,* not only enhances stress tolerance but also avoids growth defects.
Constitutive overexpression of *DREB1A* enhances stress tolerance but causes plant growth defects. However, by using a stress-responsive promoter, *RD29A,* to induce high levels of *DREB1* specifically during stress conditions, growth defects can be avoided while enhancing stress tolerance. The *DREB1A* transcription factor, which is produced in large amounts during stress conditions, can exhibit a very high level of stress tolerance by amplifying gene expression through binding of the *cis*-element DRE of the *RD29A* promoter.

3. International collaboration to improve crop drought tolerance using three genetic modifications

Based on the findings presented in the previous section, we proposed a strategy to use genetic modification to improve environmental stress tolerance in plants (**Fig. 7**). There are three points to the strategy:

(1) Promoter: an expression regulatory region upstream of a plant gene that can improve gene expression under environmental stress conditions.

(2) Gene: a plant gene that enhances tolerance to environmental stress by increasing its expression.

(3) Transformation: A transformation technique for introducing a construct by combining the appropriate promoter (1) and gene (2).

Transformation methods vary by crop and variety, and hence there is a need to work with other organizations that adopt crop transformation techniques. The developed GM crop contains plant-derived DNA fragments and should be acceptable to consumers.

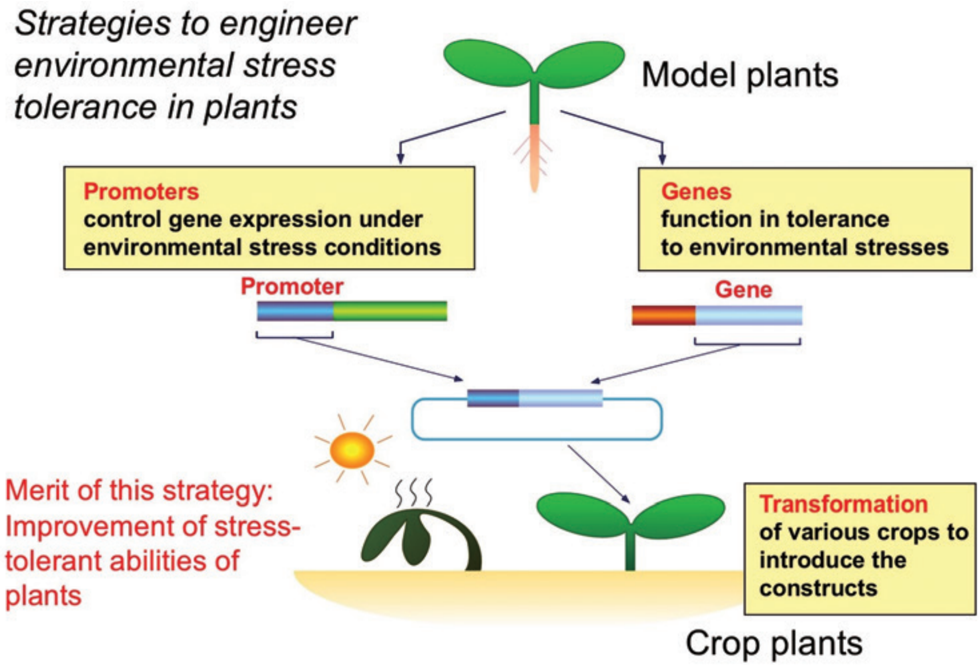

Fig. 7. Strategy for improving the environmental stress tolerance of plants using genetic modification.

Our research team has worked with research centers affiliated with the Consultative Group for International Agricultural Research (CGIAR) and national research centers in developing countries to improve the drought tolerance of some kinds of crops (**Fig. 8**; reviewed in Nakashima et al. 2014). These joint projects involve researchers from different countries specializing in diverse fields such as molecular biology, plant breeding, plant physiology, crop science, ecophysiology, and meteorology.

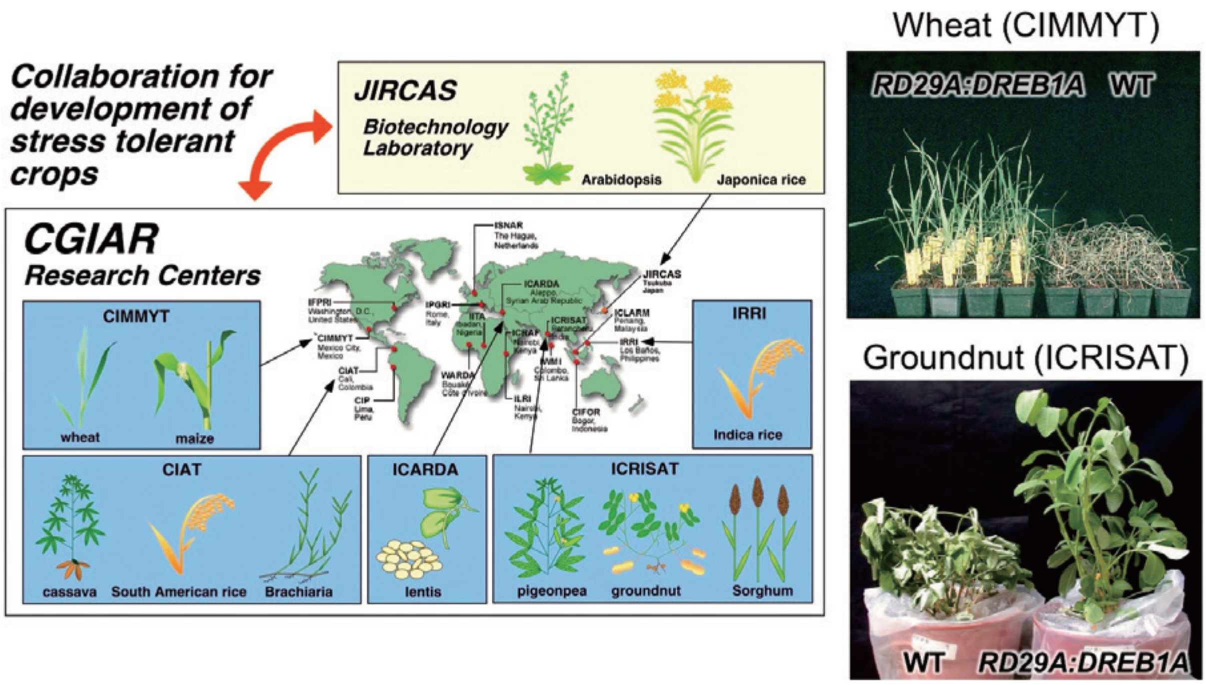

Fig. 8. International joint research with the CGIAR Research Center for the development of stress-tolerant crops.

3-1. Rice and wheat

Transgenic rice (Nipponbare) overexpressing *DREB1A* showed improved drought tolerance in greenhouses (**Fig. 9**; Ito et al. 2006). Stress-induced expression of the *DREB1A* gene in wheat delayed water stress symptoms in greenhouses (Pellegrineschi et al. 2004). Based on these results, we conducted a joint research project titled “Development of abiotic stress tolerant crops by DREB genes” (DREB Project, **Fig. 10**) supported by the Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan in 2007, for a period of five years (see **Chapter 2**). This project was carried out in collaboration with research centers affiliated with the CGIAR, such as the International Rice Research Institute (IRRI) in the Philippines, the International Tropical Agriculture Center (CIAT) in Colombia, and the International Maize and Wheat Improvement Center (CIMMYT) in Mexico (Gaudin et al. 2013, Pellegrineschi et al. 2004, Saint Pierre et al. 2012). The gene was introduced into lowland rice, upland rice, and wheat, and drought tolerance was evaluated in the field. In this project, Japanese research institutes, JIRCAS and RIKEN (The Institute of Physical and Chemical Research) produced 32 combinations of constructs using 5 promoters and 14 resistance genes, and sent them to IRRI, CIAT, and CIMMYT. Approximately 350,000 calli or embryos generated more than 1,100 independent transformation events. Grain yields of the transformants under drought conditions were investigated through tests performed in greenhouses, rainout shelters, and confined fields. From the evaluation, approximately 40 elite candidate transformants were selected. A project supported by the MAFF called "Development of Drought Tolerant Crops for Developing Countries (GM Drought Tolerance Project)" was started in 2013 for five years, and the performance of these candidate lines under drought conditions was verified. The second phase of the project, aimed at developing at least 10 elite lines from the candidates selected in the DREB project, has successfully identified several promising strains. For instance, we showed that overexpression of an *Arabidopsis thaliana* galactinol synthase gene improves drought tolerance in transgenic rice and increases grain yield in the field (Selvaraj et al. 2017). Recently, we reported that the expression of the CCCH-tandem zinc finger protein gene *OsTZF5* under a stress-inducible promoter mitigates the effect of drought stress on rice grain yield under field conditions (Selvaraj et al. 2020). In this work report, we report that the expression of the *OsNAC6* transcription factor gene under a stress-inducible promoter also mitigates the effect of drought stress on rice grain yield under field conditions (see **Chapter 2-3**).

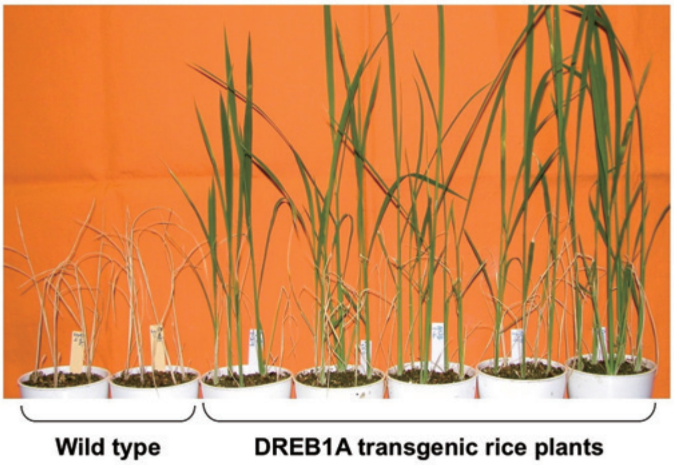

Fig. 9. Transgenic rice plants (Nipponbare) overexpressing *DREB1A* showed improved drought tolerance compared to control plants (wild type) in the greenhouse.
The figure was adapted from Ito et al. (2006).

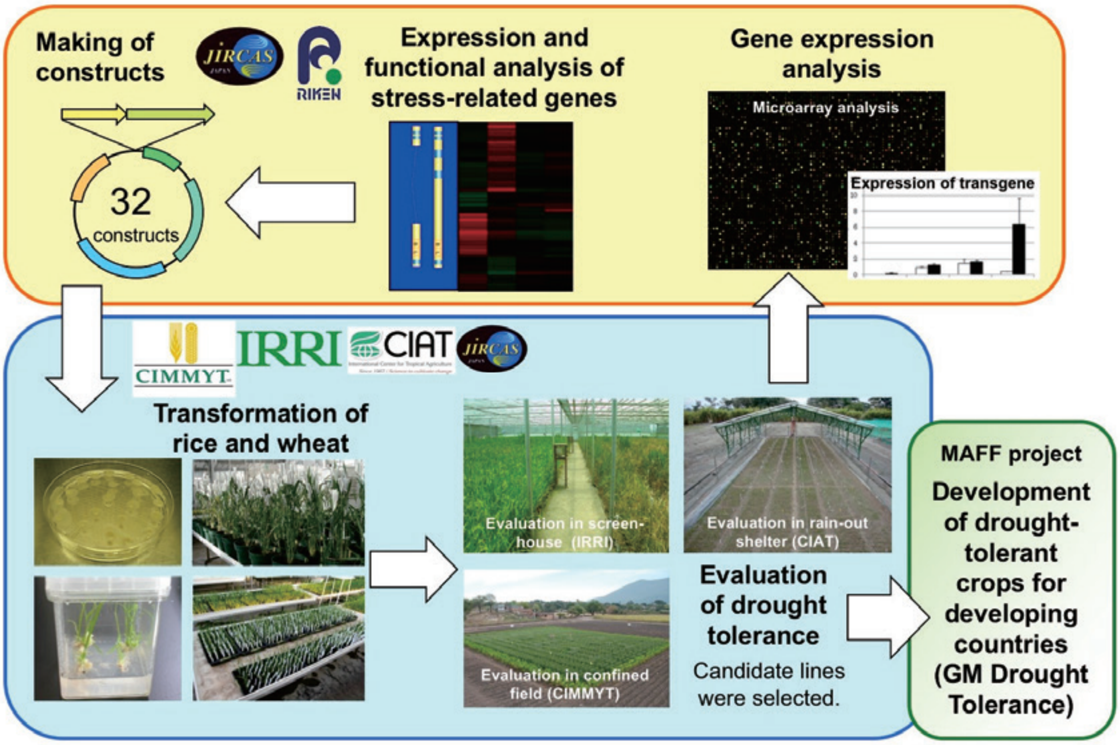

Fig. 10. Outline of the projects "Development of stress tolerant crops utilizing DREB genes" and "Development of drought-tolerant crops for developing countries" supported by the Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan.
The figure was adapted from Nakashima and Suenaga (2017).

3-2. Soybean

We showed that transgenic soybean plants expressing *RD29A:DREB1A* improved drought tolerance under greenhouse conditions (**Fig. 11**; Polizel et al. 2011). We introduced stress-tolerant genes into soybean and then evaluated drought tolerance in greenhouses and confined fields in collaboration with RIKEN, the University of Tokyo, and Embrapa (Brazilian Corporation of Agricultural Research) in the Science and Technology Research Partnership for Sustainable Development (SATREPS) project supported by the Japan Science and Technology Agency (JST) and Japan International Cooperation Agency (JICA) since 2009 (**Fig. 12**; see **Chapter 3-2**). Soybeans expressing stress-related genes such as *DREB* or *AREB* were generated to examine drought tolerance under greenhouse conditions and field trials (Barbosa et al. 2012; Engels et al. 2013; Fuganti-Pagliarini et al. 2017; Leite et al. 2014; Marinho et al. 2016; Polizel et al. 2011; Rolla et al. 2014). Researchers from Japan have found important genes involved in stress response and tolerance, such as soybean DREB and AREB transcription factors, and stress-inducible promoters (Nakashima et al. 2014; see **Chapter 3-1**). Metabolite/phytohormone–gene regulatory networks in soybean organs under dehydration conditions were revealed by integration analysis (Maruyama et al. 2020). For soybean, it has been difficult to produce transformants because of the very low transformation efficiency. However, by establishing a transformation method using *Agrobacterium*, we succeeded in improving the transformation efficiency of Brazilian soybean varieties. When using the reporter *β*-glucuronidase gene for transformation, the transformation efficiency by the established method was 1.74%. This highly efficient method has enabled the production of transgenic soybeans at a practical level. We developed 37 different transgenic lines using the particle gun or *Agrobacterium* methods. Subsequently, in greenhouses and confined fields, we evaluated their drought tolerance. During the SATREPS period, 7 of 11 lines evaluated in the greenhouse and 1 of 4 lines evaluated in the field were tolerant to drought conditions. Under water deficit conditions in the field, a better performance was observed in the *1Ea2939 AREB* line, which showed a higher performance than the wild type and other GM lines (Fuganti-Pagliarini et al. 2017). Experiments after the SATREPS project revealed that more lines were tolerant to drought (see **Chapter 3-2**). Therefore, we can expect to produce higher yields of transgenic soybean varieties under drought conditions in the future. Considering the current situation where GM soybeans are used in more than 90% of Brazil's soybean producing regions (Rally da Safra 2016) and 80% of the world's total soybean producing regions (ISAAA 2016), drought-tolerant GM soybean varieties are expected to be used not only in Brazil but also around the world.

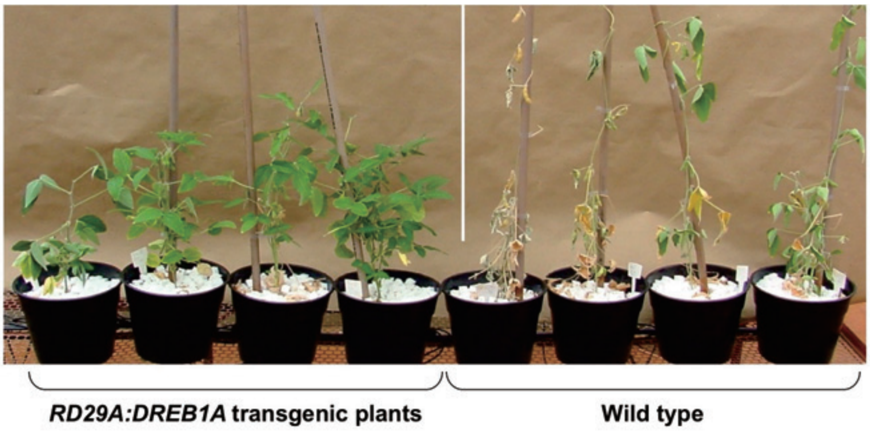

Fig. 11. Transgenic soybeans expressing *RD29A:DREB1A* showed improved drought tolerance compared to untransformed soybeans (Wild type) in a drought tolerance test performed in a greenhouse in Brazil.

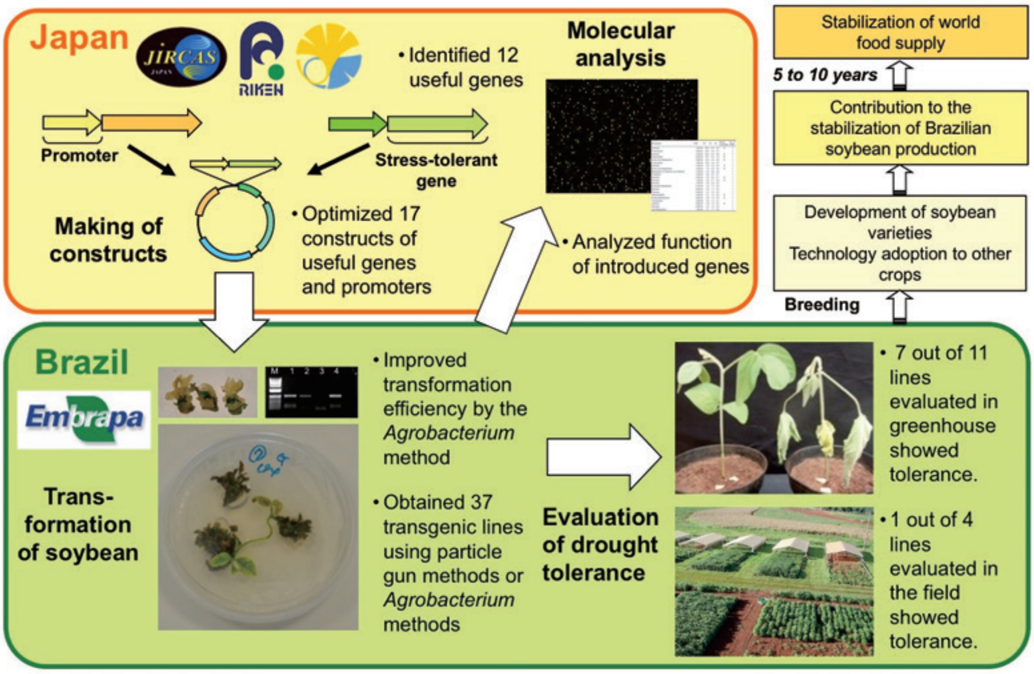

Fig. 12. To generate drought-tolerant soybean lines in Brazil, the SATREPS project for "Development of genetic engineering technology of crops with stress tolerance against degradation of global environment" has been implemented through international collaboration between Japan and Brazil.

3-3. Other crops

In collaboration with the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India, transgenic peanuts expressing *DREB1* have been developed (Bhatnagar-Mathur et al. 2007). *DREB1*-expressing peanut lines showed drought tolerance under greenhouse conditions, and their drought tolerance was confirmed by field tests (Bhatnagar-Mathur et al. 2007, 2013). Drought tests in experimental fields resulted in significant yield improvements of up to 24% (Bhatnagar-Mathur et al. 2013). The transgenic peanut lines had significantly higher seed loading values than the wild-type varieties.

In collaboration with Embrapa Agroenergy of Brazil, we introduced the *Arabidopsis DREB2Aca* gene into sugarcane (see **Chapter 3-3**). Dehydration assays using transgenic sugarcane expressing this gene under greenhouse conditions showed improved drought tolerance (Reis et al. 2014). The next study analyzed the performance of sugarcane transformants in fields under drought conditions and showed good results (de Souza et al. 2019). Sugarcane can propagate through buds and does not require genetic fixation. Therefore, commercializing genetically modified sugarcane varieties may be easier than crops that require transgene fixation.

4. Conclusion

To ensure food and nutrition safety, we have developed technologies and crops that are productive and can adapt to changing adverse environmental and climatic conditions. In order to develop stress-tolerant crops using biotechnology, we have promoted international joint research projects to develop crops such as rice, wheat, soybean, and sugarcane. Through these projects, overexpression of genes encoding stress-related transcription factors (e.g., DREB, AREB) and enzymes (e.g., galactinol synthase) of *Arabidopsis thaliana* have led to drought tolerance in GM crops such as rice, wheat, soybean, and sugarcane. There are four phases in the research and development (R&D) steps to bring GM crops to market (commercialization) after gene discovery (**Fig. 13**): Phase I, proof of concept; Phase II, early development; Phase III, advanced development; and Phase IV, pre-launch. To date, we have conducted international collaborative research projects to demonstrate proof of concept in Phase I through Phase III. We hope that the developed crops can move to the next stage of market launch and contribute to food and nutrition security in developing regions.

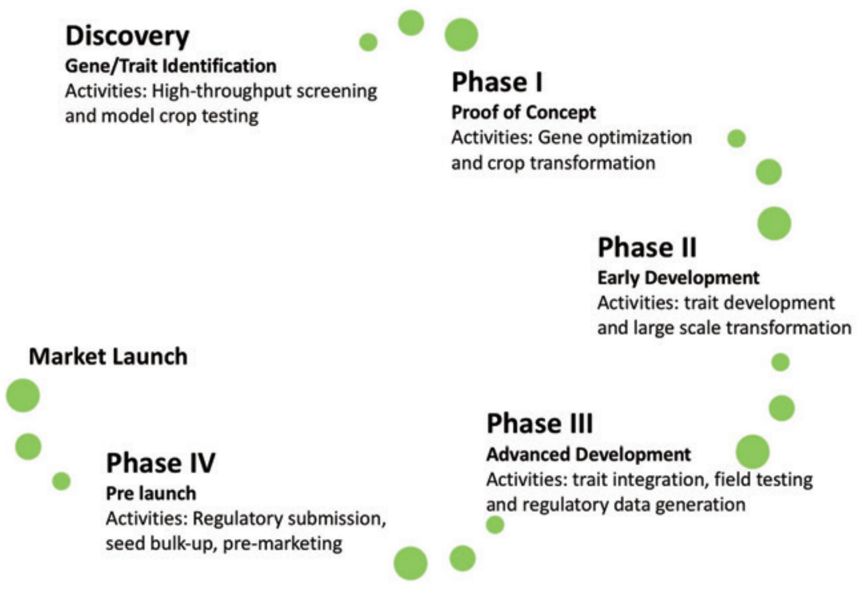

Fig. 13. Research and development steps for commercialization of GMOs.
This figure is a modified version of the original drawing of Dr. Manabu Ishitani (CIAT).

In **Chapter 2** of this work report, the achievements of an international joint research project on the development of drought-tolerant rice and wheat (the DREB Project), supported by the MAFF, Japan, will be introduced. In **Chapter 3**, the achievements of the international joint research project on the development of drought-tolerant soybean (the SATREPS Project), supported by the JST) and the JICA, and the related project on the development of drought-tolerant sugarcane will be introduced.

Acknowledgments

The international joint research project on the development of drought-tolerant rice and wheat was supported by the MAFF, Japan. The international joint research project on the development of drought-tolerant soybean was performed in the SATREPS, supported by the JST and the JICA. I thank these organizations for their support with these projects. I also express my deep appreciation to the participants of these projects. We would like to thank Editage (www.editage.com) for English language editing.

References

Barbosa EGG, Leite JP, Marin SRR, Marinho JP, Carvalho JFC, Fuganti-Pagliarini R, Farias JRB, Neumaier N, Marcelino-Guimarães FC, Oliveira MCN, Yamaguchi-Shinozaki K, Nakashima K, Maruyama K, Kanamori N, Fujita Y, Yoshida T, Nepomuceno AL (2012) Overexpression of the ABA-dependent AREB1 transcription factor from *Arabidopsis thaliana* improves soybean tolerance to water deficit. *Plant Mol Biol Report* **31**: 719-730.

Bhatnagar-Mathur P, Devi MJ, Reddy DS, Lavanya M, Vadez V, Settaj R, Yamaguchi-Shinozaki K, Sharma KK (2007) Stress-inducible expression of *At* DREB1A in transgenic peanut (*Arachis hypogaea* L.) increases transpiration efficiency under water-limiting conditions. *Plant Cell Rep* **26**: 2071-2082.

Bhatnagar-Mathur P, Rao JS, Vadez V, Dumbala SR, Rathore A, Yamaguchi-Shinozaki K, Sharma KK (2013) Transgenic peanut overexpressing the *DREB1A* transcription factor has higher yields under drought stress. *Mol Breeding* **33**: 327-340.

de Souza WR, de Oliveira NG, Vinecky F, Ribeiro AP, Basso MF, Casari RACN, da Cunha BADB, Duarte KE, Santiago TR, Martins PK, Aucique-Perez CE, Cristofoletti Júnior SC, Nepomuceno AL, de Sousa CAF, Kobayashi AK, Nakashima K, Yamaguchi-Shinozaki K, Molinari HBC (2019). Field evaluation of *AtDREB2A CA* overexpressing sugarcane for drought tolerance. *Journal of Agronomy and Crop Science* **00**:1-9. https://doi.org/10.1111/jac.12341

Engels C. Fuganti-Pagliarini R, Marin SRR, Marcelino-Guimarães FC, Oliveira MCN, Kanamori N, Mizoi J, Nakashima K, Yamaguchi-Shinozaki K, Nepomuceno AL (2013) Introduction of the *rd29A:AtDREB2A* CA gene into soybean (*Glycine max* L. Merril) and its molecular characterization in leaves and roots during dehydration. *Gen Mol Biol* **36**: 556-565.

Fuganti-Pagliarini R, Ferreira LC, Rodrigues FA, Molinari HBC, Marin SRR, Molinari MDC, Marcolino-Gomes J, Mertz-Henning LM, Farias JRB, de Oliveira MCN, Neumaier N, Kanamori N, Fujita Y, Mizoi J, Nakashima K, Yamaguchi-Shinozaki K, Nepomuceno AL. (2017) Characterization of soybean genetically modified for drought tolerance in field conditions. *Front Plant Sci* **8**: 448.

Gaudin AC, Henry A, Sparks AH, Slamet-Loedin IH (2013) Taking transgenic rice drought screening to the field. *J Exp Bot* **64**: 109-117.

Haefele SM, Nelson AD, Hijmans RJ (2014) Soil quality and constraints in global rice production. *Geoderma* **235-236**: 250-259.

ISAAA (2016) Global Status of Commercialized Biotech/GM Crops: 2016, ISAAA Briefs 52, ISAAA. https://www.isaaa.org/resources/publications/briefs/52/download/isaaa-brief-52-2016.pdf

Ito Y, Katsura K, Maruyama K, Taji T, Kobayashi M, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2006) Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. *Plant Cell Physiol* **47**: 141-153.

Iuchi S, Kobayashi M, Taji T, Naramoto M, Seki M, Kato T, Tabata S, Kakubari Y, Yamaguchi-Shinozaki

K, Shinozaki K (2001) Regulation of drought tolerance by gene manipulation of 9-*cis*-epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in *Arabidopsis*. *Plant J* **27**: 325–333.

Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K, 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.

Kim W, Iizumi T, Nishimori M (2019) Global patterns of crop production losses associated with droughts from 1983 to 2009. *Journal of Applied Meteorology and Climatology* https://doi.org/10.1175/JAMC-D-18-0174.1

Leite JP, Barbosa EG, Marin SR, Marinho JP, Carvalho JF, Pagliarini RF, Cruz AS, Oliveira MC, Farias JR, Neumaier N, Guimarães FC, Yoshida T, Kanamori N, Fujita Y, Nakashima K, Shinozaki KY, Desidério JA, Nepomuceno AL (2014) Overexpression of the activated form of the *AtAREB1* gene (*AtAREB1ΔQT*) improves soybean responses to water deficit. *Genet Mol Res* **13**: 6272-6286.

Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, 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.

Marinho JP, Kanamori N, Ferreira LC, Fuganti-Pagliarini R, Carvalho JFC, Freitas RA, Marin SRR, Rodrigues FA, Mertz-Henning LM, Farias JRB, Neumaier N, de Oliveira MCN, Marcelino-Guimarães FC, Yoshida T, Fujita Y, Yamaguchi-Shinozaki K, Nakashima K, Nepomuceno AL (2016) Characterization of molecular and physiological responses under water deficit of genetically modified soybean plants overexpressing the *AtAREB1* transcription factor. *Plant Mol Biol Rep* **34**: 410.

Maruyama K, Urano K, Kusano M, Sakurai T, Takasaki H, Kishimoto M, Yoshiwara K, Kobayashi M, Kojima M, Sakakibara H, Saito K, Shinozaki K (2020) Metabolite/phytohormone–gene regulatory networks in soybean organs under dehydration conditions revealed by integration analysis. *Plant J.* (in press) doi:10.1111/tpj.14719

Nakashima K, Yamaguchi-Shinozaki K, Shinozaki K (2014) The transcriptional regulatory network in the drought response and its crosstalk in abiotic stress responses including drought, cold, and heat. *Front Plant Sci* **5**: 170.

Nakashima K, Suenaga K (2017) Toward the genetic improvement of drought tolerance in crops. *JARQ* **51**: 1-10.

Pellegrineschi A, Reynolds M, Pacheco M, Brito RM, Almeraya R, Yamaguchi-Shinozaki K, Hoisington D (2004) Stress-induced expression in wheat of the *Arabidopsis thaliana* DREB1A gene delays water stress symptoms under greenhouse conditions. *Genome* **47**: 493–500.

Polizel AM, Medri ME, Nakashima K, Yamanaka N, Farias JR, de Oliveira MC, Marin SR, Abdelnoor RV, Marcelino-Guimarães FC, Fuganti R, Rodrigues FA, Stolf-Moreira R, Beneventi MA, Rolla AA, Neumaier N, Yamaguchi-Shinozaki K, Carvalho JF, Nepomuceno AL (2011) Molecular, anatomical and physiological properties of a genetically modified soybean line transformed with rd29A:AtDREB1A for the improvement of drought tolerance. *Genet Mol Res* **10**: 3641-3656.

Rally da Safra (2016) FINAL RESULTS Rally da Safra 2016, http://form.rallydasafra.com.br/37faf0815cc2c9f44cb2

Reis RR, da Cunha BA, Martins PK, Martins MT, Alekcevetch JC, Chalfun A Jr, Andrade AC, Ribeiro AP, Qin F, Mizoi J, Yamaguchi-Shinozaki K, Nakashima K, Carvalho Jde F, de Sousa CA, Nepomuceno AL, Kobayashi AK, Molinari HB (2014) Induced over-expression of *AtDREB2A CA* improves drought tolerance in sugarcane. *Plant Sci* **221-222**: 59-68.

Rolla AA, de Fátima Corrêa Carvalho J, Fuganti-Pagliarini R, Engels C, do Rio A, Marin SR, de Oliveira MC, Beneventi MA, Marcelino-Guimarães FC, Farias JR, Neumaier N, Nakashima K, Yamaguchi-Shinozaki K, Nepomuceno AL (2014). Phenotyping soybean plants transformed with rd29A:AtDREB1A for drought tolerance in the greenhouse and field. *Transgenic Res* **23**: 75–87.

Saint-Pierre C, Crossa JL, Bonnett D, Yamaguchi-Shinozaki K, Reynolds MP (2012) Phenotyping transgenic wheat for drought resistance. *J Exp Botany* **63**: 1799-1808.

Selvaraj MG, Ishizaki T, Valencia M, Ogawa S, Dedicova B, Ogata T, Yoshiwara K, Maruyama K, Kusano M, Saito K, Takahashi F, Shinozaki K, Nakashima K, 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 Biotech J* **15**:1465-1477. doi: 10.1111/pbi.12731

Selvaraj MG, Jan A, Ishizaki T, Valencia M, Dedicova B, Maruyama K, Ogata T, Todaka D, Yamaguchi-Shinozaki K, Nakashima K, Ishitani M (2020) Expression of the CCCH-tandem zinc finger protein gene *OsTZF5* under a stress-inducible promoter mitigates the effect of drought stress on rice grain yield

under field conditions. *Plant Biotech J* (in press) doi: 10.1111/pbi.13334
Taji T, Ohsumi C, Iuchi S, Seki M, Kasuga M, Kobayashi M, Yamaguchi-Shinozaki K, Shinozaki K (2002) Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana. Plant J* **29**:417-426.
United Nations Information Centre (2018) https://sustainabledevelopment.un.org/sdgs
World Food Programme (2018) http://cdn.wfp.org/down/