REVIEW
Toward the Genetic Improvement of Drought Tolerance in Crops

Kazuo NAKASHIMA[1]* and Kazuhiro SUENAGA[2]

[1] Stable Agricultural Production Program, Japan International Research Center for Agricultural Sciences
   (Tsukuba, Ibaraki 305-8686, Japan)
[2] Tropical Agriculture Research Front, Japan International Research Center for Agricultural Sciences
   (Ishigaki, Okinawa 907-0002, Japan)

Abstract

Crop damage due to environmental stresses, including drought, high salinity, and high temperature, occurs worldwide. Therefore, genetically modifying plants to increase their environmental stress tolerance is an important global issue. In this paper, we discuss recent developments in basic and applied research aimed at genetically improving crop environmental stress tolerance. First, we review the progress made in understanding the environmental stress-tolerance mechanisms in plants, using *Arabidopsis* or rice as models. Then, we discuss our international collaboration to genetically modify crops such as rice, wheat, sugarcane and soybean that entails greenhouse- or field-based drought-tolerance tests. Finally, we assess the future prospects for developing stress-tolerant varieties.

Introduction

Extreme weather occurs frequently around the world, causing crop damage. The damage caused by droughts is extensive and has become a serious global problem (Yadav et al. 2011). Drought, which leads to extensive damage over several decades, has caused a significant loss in food production. In 2012, droughts in the United States affected the production of such crops as maize and soybean, and had a huge impact on the world's food supply (Edmeades 2013, Nakashima et al. 2014). In Brazil, droughts often damage the production of soybean (Nakashima et al. 2014), and severe droughts have occurred there four times (2004/2005, 2008/2009, 2011/2012, and 2013/2014 seasons) in the past 10 years (Alexandre Nepomuceno, Embrapa, Brazil, personal communication). Drought is a major cause of hunger in developing countries. Of the total rice cultivation area in Asia, rain-fed fields account for 20%, totaling 23 million hectares, and are vulnerable to the impact of drought (Pandey & Bhandari 2007). The United Nations predicts that 2.7 billion people will face severe water shortages in 2025. Given current population growth and possible water shortages in the near future, the *Comprehensive Assessment of Water Management in Agriculture* released in 2003 by the International Water Management Institute concluded that it is necessary to increase crop production by 40% in drought areas before 2025 (Pennisi 2008). Thus, drought-tolerant crops such as rice, wheat, maize, and soybean must be developed to ensure food security.

We have studied the molecular mechanisms involved in environmental stress responses in rice and *Arabidopsis* as model plants for more than 20 years, and have revealed that stress-inducible transcription factors (TFs) such as dehydration-responsive element-binding protein (DREB), abscisic acid (ABA)-responsive element-binding factor (AREB), NAM (no apical meristem), ATAF (*Arabidopsis* transcription activation factor), and CUC (cup-shaped cotyledon) (NAC) play important roles in regulating stress responses and tolerances (Fig. 1; reviewed in Nakashima et al. 2009, Nakashima et al. 2014, Yamaguchi-Shinozaki & Shinozaki 2006). The overexpression of these key genes enhanced stress tolerance in rice and *Arabidopsis* in greenhouse experiments. We have been collaborating with various research institutes to examine whether such genes can improve stress tolerance in the field or in other crops (reviewed in Nakashima et al. 2014). This review provides
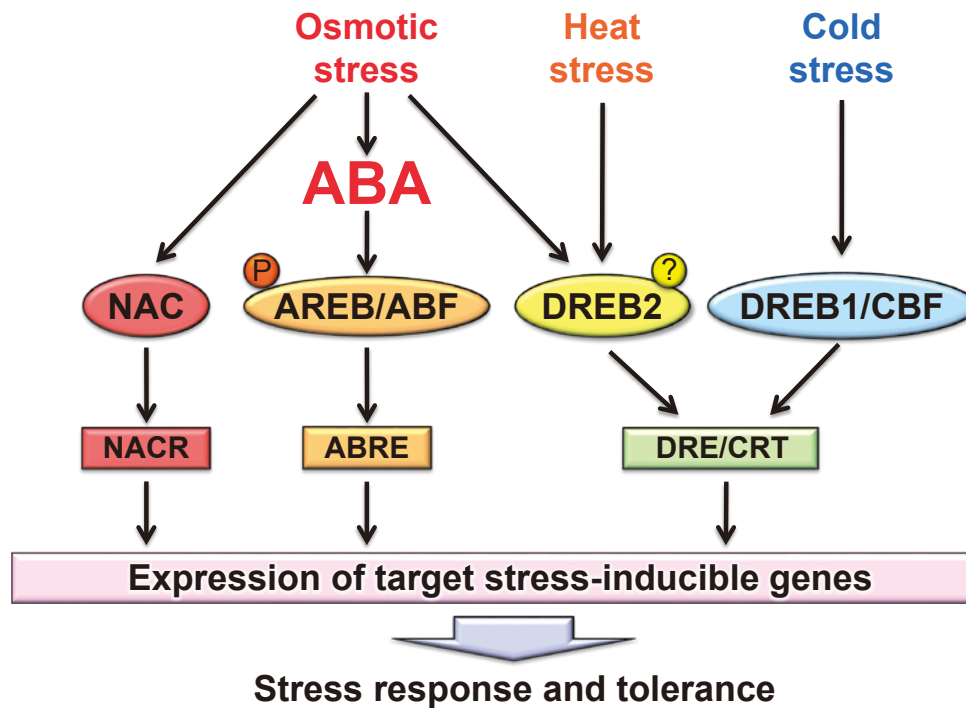
Fig. 1. Transcriptional network under environmental stress conditions in plants.
Abiotic stresses such as osmotic stress, heat stress, and cold stress induce the expression and/or activation of transcription factors. The transcription factors bind to specific *cis*-elements to induce the expression of targeted stress-inducible genes. The products of the genes function in stress response and tolerance. Ellipses denote transcription factors; boxes denote *cis*-elements. Details are described in the text.

an overview of the recent progress made in molecular studies on stress tolerance and the current status of international collaborative research on improving stress tolerance in crops.

Molecular responses and tolerance to stresses in plants

1. DREB1/CBF TFs

DREB protein 1A (DREB1A), DREB1B, and DREB1C are plant-specific AP2/ERF-type TFs which were isolated as proteins that bind to DREs in promoters responsive to drought, high salinity, and low temperature in *Arabidopsis* (Liu et al. 1998). These genes are located in a locus in tandem and are induced by low temperatures. The DREB1s were also isolated as C-repeat binding factors (CBFs) that bind to C-repeats in cold-responsive promoters (Jaglo-Ottosen et al. 1998). Recent studies have revealed that natural variations in the CBFs' cold-response pathway correlate with local adaptations in *Arabidopsis* ecotypes (Gehan et al. 2015). DREB-type proteins have been isolated from a number of other plant species such as barley, rice, sunflower, maize, and wheat (reviewed in Mizoi et al. 2012).

Overexpression of these genes enhanced the tolerance to drought, high salinity, and low temperature in *Arabidopsis*. In transgenic plants including rice and tobacco, overexpressing DREB1A also showed improved drought tolerance under greenhouse conditions (Ito et al. 2006, Kasuga et al. 2004). However, the constant overexpression of DREB1A using the *35S* promoter of the Cauliflower Mosaic Virus induced growth defects. Such stress-responsive promoters as *RD29A* of *Arabidopsis* are associated with the avoidance of growth defects caused by DREB1A expression (Kasuga et al. 1999).

2. DREB2 TFs

DREB2s were also isolated as DREB proteins (Liu et al. 1998). However, the overexpression of DREB2 did not induce expression of the target genes, indicating that modifications are necessary to activate the DREB2 proteins. DREB2Aca, which harbors a defect in its negative regulatory domain, is transcriptionally active and the stress-responsive overexpression of DREB2Aca improved stress tolerance in *Arabidopsis* (Sakuma et al. 2006a). An integrated analysis of transcripts and metabolites showed very different expression levels of the genes responsible for

carbohydrate metabolism in DREB1A and DREB2A transgenic *Arabidopsis* (Maruyama et al. 2009). DREB proteins, including DREB2A, cooperate with AREB/ABF proteins in gene expression (Lee et al. 2010, Narusaka et al. 2003). AREB/ABFs and SNF1-related protein kinases type 2s (SnRK2s) are involved in expression of the *DREB2A* gene under osmotic stress conditions (Kim et al. 2011), suggesting that there are complex interactions between the AREB and DREB pathways. We will describe the international collaborative research focused on developing drought-tolerant plants using DREB2. The function of DREB2 during heat stress is described next.

Heat and the accompanying dehydration cause severe damage to crops in drought situations. Heat shock factors are important TFs for controlling the expression of heat shock proteins in both flora and fauna. A comprehensive microarray analysis of the transgenic plants overexpressing DREB2 revealed that DREB2 induces the expression of heat-related genes, such as heat shock proteins. The overexpression of DREB2Aca can enhance the tolerance levels to drought and heat stress in *Arabidopsis* (Sakuma et al. 2006b), indicating that the overexpression of DREB2 might improve the heat and drought tolerance of crops in the field. Recent studies showed that DNA polymerase II subunit B3-1 (DPB3-1)/nuclear factor Y, subunit C10 (NF-YC10) binds to DREB2A, and the overexpression of DPB3-1 improves heat tolerance in *Arabidopsis* (Sato et al. 2014). Dpb3-1

might be useful in improving the heat tolerance of crops. Heat shock factors control *DREB2A* expression, suggesting that there are cascades of TFs involved in controlling gene expression under heat-stress conditions (Yoshida et al. 2011).

3. AREB1/ABF TFs

ABA is a phytohormone that plays important roles in plants under water-limiting conditions such as drought. ABA controls molecular responses, including the expression of genes associated with drought response and tolerance (Fig. 1), and mediates physiological responses, including stomatal closure. AREB/ABFs are ABA-responsive bZIP-type TFs. These bind to ABREs in the stress-responsive promoters and activate gene expression (Figs. 1, 2). Among the AREB/ABF TFs in *Arabidopsis*, AREB1, AREB2, and ABF3 were reported to have important roles in ABA signaling in the vegetative stage under drought conditions (Yoshida et al. 2010). Recently, ABF1 was also reported to play an important role in ABA signaling and drought tolerance in the vegetative stage in *Arabidopsis* (Yoshida et al. 2015). Other AREB/ABF TFs, including ABI5 and EEL, were reported to have roles in seed maturation and germination (reviewed in Fujita et al. 2013).

Because the native form of *AREB1* cannot activate target genes such as *RD29B* in *Arabidopsis*, modification is necessary for transcriptional activation. Our group revealed
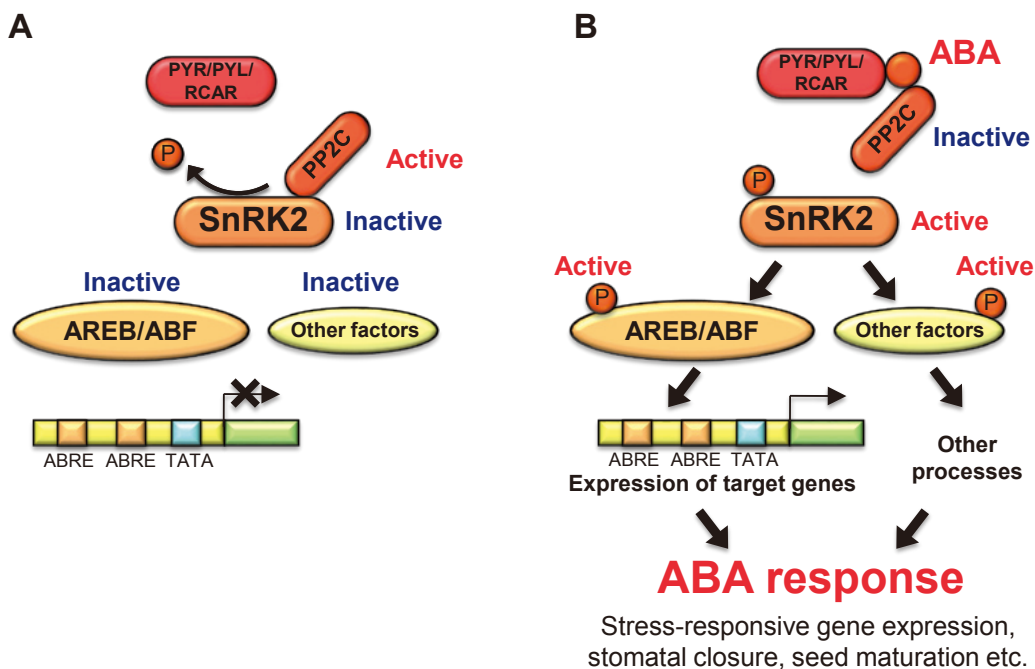

Fig. 2. Model of ABA perception and signaling in plants.
(A) Under normal growth conditions; (B) Under stress conditions or during seed maturation.

that the deletion (Fujita et al. 2005) or phosphorylation-mimic (Furihata et al. 2006) forms of AREB1 could activate transcription, and that the overexpression of such forms could also improve drought tolerance.

In ABA research, ABA PYR/PYL/RCAR receptors have recently been discovered (reviewed in Cutler et al. 2010, Fujita et al. 2013a, 2013b, Miyakawa et al. 2013, Nakashima and Yamaguchi-Shinozaki 2013, Raghavendra et al. 2010, Umezawa et al. 2010, Weiner et al. 2010). Figure 2 shows the ABA perception and signaling mechanisms. When ABA does not exist (stress-free condition; Fig. 2 A), type 2C protein phosphatases (PP2Cs) dephosphorylate SnRK2s, resulting in inactivation of the SnRK2s and ABA signal transduction not occurring. When ABA exists (stress conditions and seed maturation stage: Fig. 2 B), it forms a complex with the PYR/PYL/RCAR receptors and PP2Cs, inactivating the PP2Cs. Then SnRK2 can phosphorylate target proteins such as AREB/ABFs TFs; consequently, AREB/ABFs are activated. The activated AREB/ABFs bind to ABRE in stress-responsive promoters and trigger gene expression. The SnRK2-activated proteins and the expressed proteins function in ABA responses. ABA receptors form a family in plants, and the differences in their expression patterns and functions are under study.

PP2Cs in Clade A, including ABI1 and ABI2, are negative regulators of ABA signaling and play important roles in ABA perception and signaling as described above (Fig. 2). This clade contains ~10 PP2Cs in rice and *Arabidopsis*, but their expression patterns and functions are not well known. Interestingly, recent studies have suggested that ABI2 modulates nitrate sensing and uptake (Léran et al. 2015), and that ABI1 regulates the signaling involved in the carbon and nitrogen nutrient balance (Lu et al. 2015). Crosstalk between ABA and nutrient signaling could possibly control plant growth under stress conditions.

SnRK2s are positive regulators in ABA signaling and play important roles in controlling the activity of target proteins, including AREB/ABFs, through phosphorylation, as described above (Fig. 2). We studied three types of ABA-associated SnRK2s (SRK2D, SRK2E, and SRK2I) in *Arabidopsis* and revealed their crucial roles in ABA signaling (Fujita et al. 2009, Nakashima et al. 2009a). The triple knockout mutant showed defects in all of the traits involved in ABA response, such as ABA-responsive gene expression, drought tolerance, and seed dormancy. The data suggest that these SnRK2s function in the control of stress tolerance in the vegetative stage, and in the control of seed maturation and germination.

4. Other TFs

Other TFs, including MYB, MYC, and NAC, are also related to stress response and tolerance in *Arabidopsis* (reviewed in Nakashima et al. 2009b, Nakashima et al. 2014, Yamaguchi-Shinozaki & Shinozaki 2006). We studied stress-responsive NACs in *Arabidopsis* and rice, and showed that the overexpression of NACs improves stress tolerance (Nakashima et al. 2007, Nakashima et al. 2013, Takasaki et al. 2010, Tran et al. 2004). Other groups also reported that the overexpression of NACs can improve drought tolerance. For instance, the overexpression of SNAC1 in rice improves drought tolerance in the field (Hu et al. 2006). NACs may be involved in root growth (Jeong et al. 2013, Redillas et al. 2012), and a recent study revealed that stress-responsive NACs affect the control of senescence (Garapati et al. 2015, Takasaki et al. 2015). Takasaki et al. (2015) identified the major roles of seven genes from the A subfamily of stress-responsive NACs (*SNAC-A*) in ABA-inducible leaf senescence during stress responses. An analysis of the septuple mutant revealed the retardation of ABA-inducible leaf senescence. They also identified target genes of the SNAC-A TFs to show the differences between AREB/ABF-inducible genes and SNAC-A-inducible genes. These results suggest that the *SNAC-A* subfamily of genes is mainly involved in ABA-induced leaf senescence.

5. Other factors

Genes encoding proteins other than TFs that have important functions in stress-response and tolerance have also been reported (reviewed in Nakashima et al. 2009b, Nakashima et al. 2014, Yamaguchi-Shinozaki & Shinozaki 2006). For instance, genes encoding 9-*cis*-epoxycarotenoid dioxygenase (NCED) to synthesize ABA (Iuchi et al. 2001), galactinol synthase (GolS) for the synthesis of oligosaccharides (Taji et al. 2002), late embryogenesis abundant (LEA) proteins (Xiao et al. 2007), and CCCH-type zinc finger proteins for the control of RNA metabolism (Jan et al. 2013), have important roles in the regulation of stress tolerance. The overexpression of OsLEA3 improves the drought tolerance of rice in the field (Xiao et al. 2007). Recent studies revealed that stress-responsive OsTZF1 was involved in the control of senescence (Jan et al. 2013). While OsTZF1 is stress responsive, its constant overexpression causes growth retardation. For the purpose of crop improvement, stress-responsive promoters must be used to drive OsTZF1 to avoid growth retardation.

International collaboration for the genetic improvement of drought tolerance in crops

The frequency and severity of droughts have increased in recent years, and the resulting damage caused has become more serious as compared with previous eras. Rain-fed cultivation is impacted more greatly than irrigated cultivation, and such rain-fed cultivation areas are closely linked with areas of poverty. Thus, drought has a significant impact on social issues in developing countries. Our

research team has established international collaborative research projects in cooperation with other research institutions, including international institutes, under the umbrella of national research centers in developing countries and the Consultative Group for International Agricultural Research (CGIAR) to improve drought tolerance in crops (reviewed in Nakashima et al. 2014). In our joint projects, researchers in different countries and specializing in such diverse fields as molecular biology, transformation technology, plant breeding, plant physiology, and eco-physiology work toward the development of drought-tolerant crops.

1. Rice and wheat

We introduced such genes as *DREB1* into lowland rice, upland rice, and wheat to evaluate drought tolerance in fields 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, respectively (Gaudin et al. 2013, Pellegrineschi et al. 2004, Saint Pierre et al. 2012). Japan's Ministry of Agriculture, Forestry and Fisheries (MAFF) supported a

collaborative research project from 2007 to 2012 known as the "Development of abiotic stress tolerant crops by DREB genes" project (DREB Project, Fig. 3). The Japanese research institutes, Japan International Research Center for Agricultural Sciences (JIRCAS) and RIKEN (Institute of Physical and Chemical Research, Japan) produced 32 combinations of constructs using five promoters and 14 tolerance genes, and sent them to the IRRI, CIAT, and CIMMYT during this project. From ~350,000 calli or embryos, more than 1,100 independent transformation events were produced. The grain yields of the transformants under drought conditions were investigated through tests conducted in greenhouses, rain-out shelters, and confined fields. From the evaluation, ~40 elite candidate transformants were selected. A new project called the "Development of drought-tolerant crops for developing countries (GM Drought Tolerance project)" supported by MAFF was launched in 2013 to verify the performance of these candidates under drought conditions. The new project aims to develop at least 10 elite lines from the candidates selected in the DREB project.

2. Soybean

In collaboration with Embrapa (Brazilian Corporation

MAFF Project
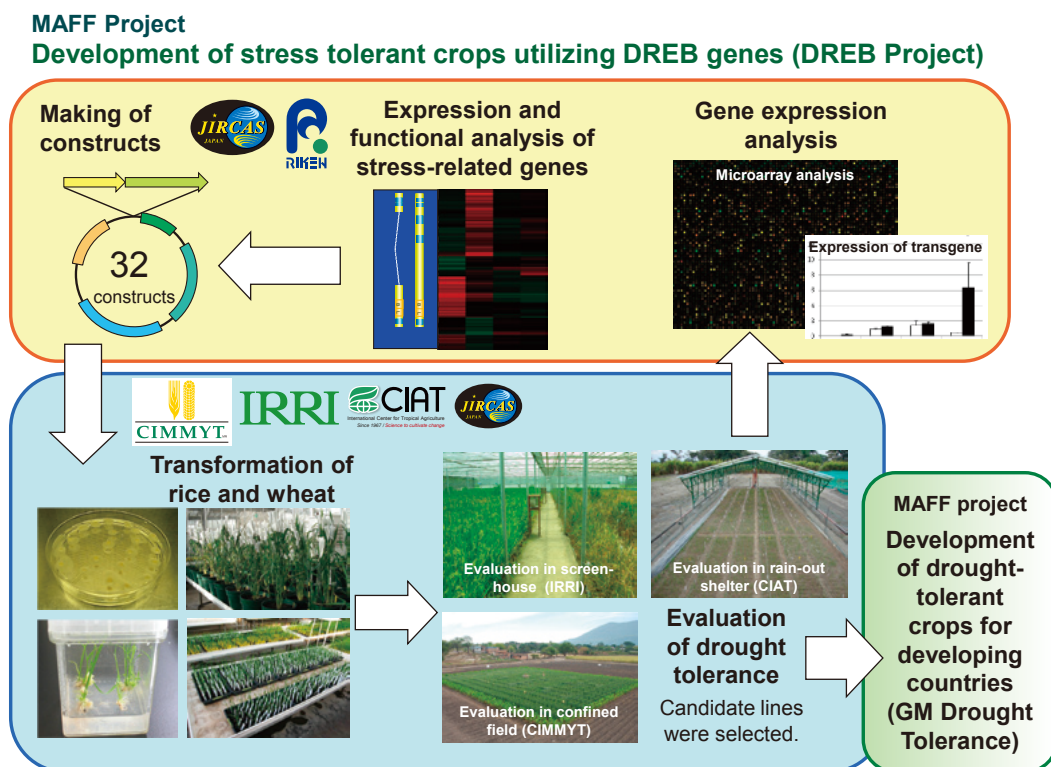**Development of stress tolerant crops utilizing DREB genes (DREB Project)**

Fig. 3. Outline of the Ministry of Agriculture, Forestry and Fisheries (MAFF, Japan) projects "Development of stress tolerant crops utilizing DREB genes" and "Development of drought-tolerant crops for developing countries."

of Agricultural Research), RIKEN, and the University of Tokyo, we introduced stress-tolerant genes into soybean and then evaluated drought tolerance in greenhouses and confined fields. This project has been supported by the Science and Technology Research Partnership for Sustainable Development of the Japan Science and Technology Agency (JST)/Japan International Cooperation Agency (JICA) since 2009 (Fig. 4). Soybeans expressing *DREB* or *AREB* genes were generated to show improved tolerance under greenhouse conditions, and we are currently conducting field trials (Barbosa et al. 2012, de Palva Rolla et al. 2013, Engels et al. 2013, Leite et al. 2014, Marinho et al. 2015, Polizel et al. 2011). For soybean, it has been difficult to produce transformants due to very low transformation efficiency. However, we succeeded in improving the transformation efficiency of Brazilian soybean cultivars by establishing a transformation method using Agrobacterium. The transformation efficiency was 1.74% when we used the reporter *β-glucuronidase* gene for transformation. This efficiency enables the production of transgenic soybeans at a practical level. We obtained 37 transgenic lines by using particle gun or *Agrobacterium* methods. Then drought tolerance was evaluated in the greenhouse and fields. Seven out

of 11 lines evaluated in the greenhouse showed tolerance, and one out of four lines evaluated showed tolerance under drought conditions in the field. Thus, we can expect to produce transgenic soybean varieties with high yields under drought conditions in the future. Given the current situation where genetically modified (GM) soybean is used in more than 90% of Brazil's soybean production area (Rally da Safra, 2016) and in 80% of the world's total soybean production area (ISAAA 2014), a GM soybean variety showing improved drought tolerance could be used worldwide, and not just in Brazil.

3. Other crops

In collaboration with the International Crop Research Institute for the Semi-Arid Tropical (ICRISAT) in India, we successfully produced *DREB1* transgenic peanuts. The peanut-expressing *DREB1* showed drought tolerance under greenhouse conditions, and its drought tolerance was confirmed in field trials (Bhatnagar-Mathur et al. 2007, 2013). A substantial yield improvement of up to 24% in field drought tests was achieved and thus related to higher harvest indices (Bhatnagar-Mathur et al. 2013). The transgenic lines had significantly higher seed-filling values
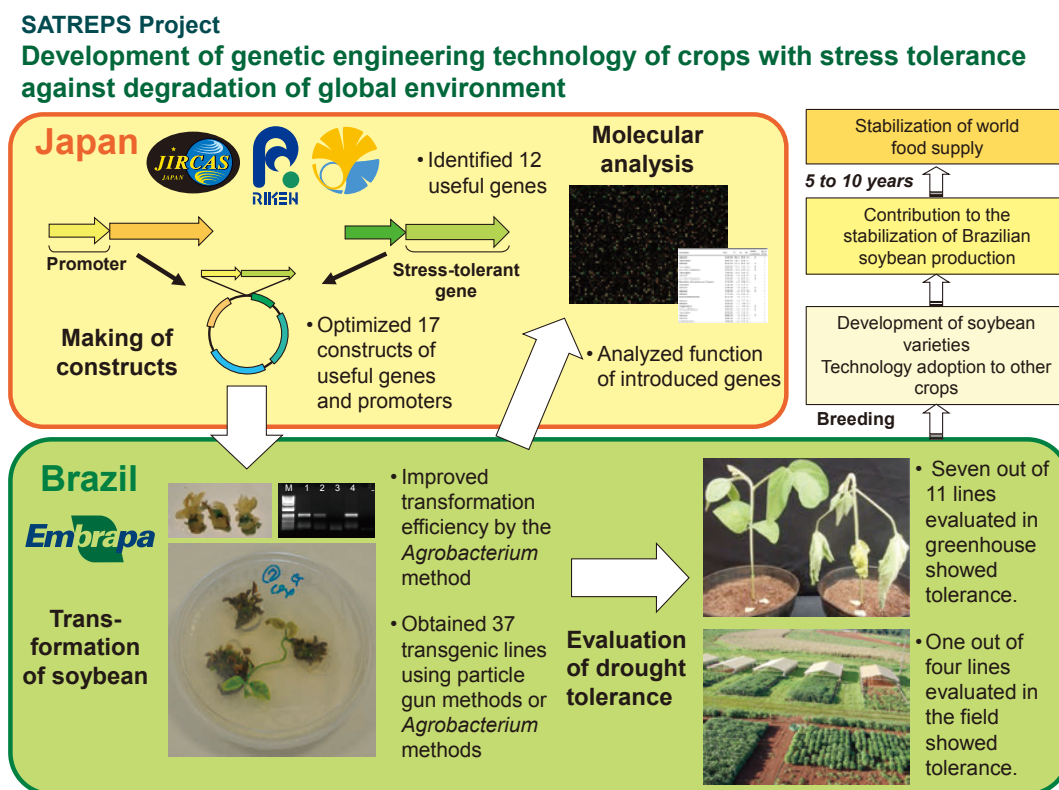

SATREPS Project
Development of genetic engineering technology of crops with stress tolerance against degradation of global environment

Fig. 4. Outline of the Science and Technology Research Partnership for Sustainable Development (SATREPS) project "Development of genetic engineering technology of crops with stress tolerance against degradation of global environment."

than the wild-type variety and displayed 20-30% pod yield reductions under drought conditions.

In collaboration with Embrapa Agroenergy in Brazil, we introduced the *DREB2* gene into sugarcane. Assays on transgenic sugarcane expressing the *DREB2Aca* gene of *Arabidopsis* also produced good results under greenhouse conditions (Reis et al. 2014). The next studies will entail analyzing the performance of sugarcane transformants in fields under drought conditions. Because sugarcane can be propagated through shoots, its genetic fixation is not required. Therefore, the development of genetically modified sugarcane varieties might be easier than in crops that require transgene fixation.

Future work

1. Promotion of research on improving drought tolerance

In collaboration with international institutions, we have shown that some drought-tolerant genes can improve the performance of crops under drought conditions as verified by tests conducted in a greenhouse. This finding suggests that such genes can work in different genetic backgrounds (plant species and/or varieties), growth stages, and environmental conditions. However, given the various drought factors such as the length and severity of a drought, soil water depth, and temperature, we must conduct more experiments. At the same time, we should select appropriate varieties, such as commercial varieties, in which to introduce these genes. As transformation efficiency differs among the varieties, it is often difficult to introduce a gene directly into practical varieties. Thus, the transgene needs to be transferred to practical varieties through crossing when the selected varieties cannot be used in transformations.

There are four types of drought-resistance mechanisms in plants: (1) drought avoidance, such as getting water from the soil by deep rooting, (2) drought escape, whereby the flowering time avoids the drought season, (3) drought tolerance, which maintains the intracellular water potential, and (4) drought recovery, whereby growth recovers after a drought (Gowda et al. 2011). Our research group has studied genes mainly related to drought tolerance. Of the other mechanisms, deep rooting is regarded as an important trait to maintain crop production under drought conditions (Yoshida & Hasegawa, 1982). Recently, the *DEEPER ROOTING1* gene (*DRO1*) was isolated in rice (Uga et al. 2013). Control of the root system's architecture by *DRO1* increased rice yield under drought conditions. Even if there is a deep-rooting gene in rice, plants may wither before the roots reach groundwater under drought conditions. Drought-tolerance genes might be effective in enabling plants to withstand the stress until rain can be received. Therefore, the combination of a drought-tolerance gene

and genes for the other resistance mechanisms might prove beneficial.

2. Impediments to commercialization

The development of GM crops entails a number of patents for genes and promoters, including the marker genes in a vector, as well as patents related to transformation techniques. Thus, it is important to implement the freedom-to-operate (FTO) surveys on patents as soon as possible after launching a project aimed at developing GM crops. Therefore, cooperation is necessary between the departments in charge of intellectual property and the research sections.

Huge amounts of funding are also needed for the development of GM crops, including for deregulation and safety assessments. It is estimated to cost an average of USD 136 million over 13 years to bring a new GM crop product to the commercial market (Prado et al. 2014). Thus, only a small number of multinational companies can currently afford to develop GM crops, and it is problematic to sustain funding for public and semi-public institutions. As drought is a major problem in developing countries, we hope that public or private funding will support the development of drought-tolerant varieties.

Conclusion

Recent molecular studies have revealed some important genes, including genes encoding DREB and AREB/ ABF TFs for stress tolerance, and that the overexpression of such genes can enhance the stress tolerance in several types of plants in a greenhouse. International collaborative research showed that transgenic plants containing such genes could improve the grain yields of such crop plants as rice and soybean under drought conditions in the field. We hope that these results will contribute to the development of commercial transgenic varieties. In order to address global food and environmental problems, a multinational framework with more interdisciplinary cooperation is needed. Furthermore, the future commercialization of GM crops requires appropriate project management, encompassing science, technology and intellectual property, and GM regulation. We hope that the crops developed in our international joint research projects may contribute to stabilizing agricultural production and providing a sufficient worldwide food supply in the future.

Acknowledgments

References

Barbosa, E. G. G. et al. (2012) Overexpression of the ABA-dependent AREB1 transcription factor from *Arabidopsis thaliana* improves soybean tolerance to water deficit. *Plant Molecular Biology Reporter*, **31**(3), 719-730.

Bhatnagar-Mathur, P., et al. (2007) Stress-inducible expression of *At* DREB1A in transgenic peanut (*Arachis hypogaea* L.) increases transpiration efficiency under water-limiting conditions. *Plant Cell Rep*, **26**(12), 2071-82.

Bhatnagar-Mathur, P. et al. (2013) Transgenic peanut overexpressing the *DREB1A* transcription factor has higher yields under drought stress. *Mol Breeding*, **33**, 327-340.

Clive James (2014) 2014 ISAAA Report on global status of biotech/GM crops. ISAAA Brief 49-2014: Slide & Tables. http://isaaa.org/resources/publications/briefs/49/pptslides/default.asp

Cutler, S. R. et al. (2010) Abscisic acid: emergence of a core signaling network. *Annu Rev Plant Biol*, **61**, 651-79.

Datta, K. et al. (2012) Overexpression of *Arabidopsis* and rice stress genes' inducible transcription factor confers drought and salinity tolerance to rice. *Plant Biotechnol J*, **10**(5), 579-86.

de Paiva Rolla, A. A. et al. (2013) Phenotyping soybean plants transformed with rd29A:AtDREB1A for drought tolerance in the greenhouse and field. *Transgenic Res*, **23**, 75-87.

Edmeades, G. O. (2013) Progress in achieving and delivering drought tolerance in maize - An update. Ithaca, NY, ISAAA. http://www.plantstress.com/RepresentPoppup.asp?Category=1&IDNUM=1420

Engels, C. et al. (2013) Introduction of the rd29A: AtDREB2A CA gene into soybean (*Glycine max* L. Merrill) and its molecular characterization in leaves and roots during dehydration. *Genetics and Molecular Biology*, **36**(4), 556-565.

Fujita, Y. et al. (2005) AREB1 is a transcription activator of novel ABRE-dependent ABA signaling that enhances drought stress tolerance in *Arabidopsis*. *Plant Cell*, **17**(12), 3470-88.

Fujita, Y. et al. (2009) Three SnRK2 protein kinases are the main positive regulators of abscisic acid signaling in response to water stress in *Arabidopsis*. *Plant Cell Physiol*, **50**(12), 2123-32.

Fujita, Y. et al. (2013) Pivotal role of the AREB/ABF-SnRK2 pathway in ABRE-mediated transcription in response to osmotic stress in plants. *Physiol Plant*, **147**(1), 15-27.

Fujita, Y. et al. (2013) Role of Abscisic Acid Signaling in

Drought Tolerance and Preharvest Sprouting Under Climate Change. In *Climate Change and Abiotic Stress Tolerance*, eds. Tuteja, N. and Gill, S. S., Wiley-Blackwell, 521-553.

Furihata, T. et al. (2006) Abscisic acid-dependent multisite phosphorylation regulates the activity of transcription activator AREB1. *Proc Natl Acad Sci USA*, **103**(6), 1988-93.

Garapati, P. et al. (2015) Transcription Factor ATAF1 in *Arabidopsis* Promotes Senescence by Direct Regulation of Key Chloroplast Maintenance and Senescence Transcriptional Cascades. *Plant Physiol*, **168**(3), 1122-39.

Gaudin, A. C. et al. (2013) Taking transgenic rice drought screening to the field. *J Exp Bot*, **64**(1), 109-17.

Gehan, M. A. et al. (2015) Natural variation in the C-Repeat Binding Factor (CBF) cold response pathway correlates with local adaptation of *Arabidopsis* ecotypes. *Plant J*, **84**, 682-693.

Gowda, V. R. P. et al. (2011) Root biology and genetic improvement for drought avoidance in rice. *Field Crops Research*, **122**(1), 1-13.

Hu, H. et al. (2006) Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. *Proc Natl Acad Sci USA*, **103**(35), 12987-92.

Ishizaki, T. et al. (2013) Expression of *Arabidopsis* DREB1C improves survival, growth, and yield of upland New Rice for Africa (NERICA) under drought. *Mol Breeding*, **31**, 255-64.

Ito, Y. et al. (2006) Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. *Plant Cell Physiol*, **47**(1), 141-53.

Iuchi, S. et al. (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**(4), 325-33.

Jaglo-Ottosen, K. R. et al. (1998) *Arabidopsis CBF1* overexpression induces *COR* genes and enhances freezing tolerance. *Science*, **280**(5360), 104-6.

Jan, A. et al. (2013) OsTZF1, a CCCH-tandem zinc finger protein, confers delayed senescence and stress tolerance in rice by regulating stress-related genes. *Plant Physiol*, **161**(3), 1202-16.

Jeong, J. S. et al. (2010) Root-specific expression of *OsNAC10* improves drought tolerance and grain yield in rice under field drought conditions. *Plant Physiol*, **153**(1), 185-97.

Jeong, J. S. et al. (2013) *OsNAC5* overexpression enlarges root diameter in rice plants leading to enhanced drought tolerance and increased grain yield in the field. *Plant Biotechnol J*, **11**(1), 101-14.

Kasuga, M. et al. (1999) Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nat Biotechnol*, **17**(3), 287-91.

Kasuga, M. et al. (2004) A combination of the *Arabidopsis* DREB1A gene and stress-inducible *rd29A* promoter improved drought- and low-temperature stress tolerance in

tobacco by gene transfer. *Plant Cell Physiol*, **45**(3), 346-50.

Kim, J. S. et al. (2011) An ABRE promoter sequence is involved in osmotic stress-responsive expression of the *DREB2A* gene, which encodes a transcription factor regulating drought-inducible genes in *Arabidopsis*. *Plant Cell Physiol*, **52**(12), 2136-46.

Lee, S. J. et al. (2010) DREB2C interacts with ABF2, a bZIP protein regulating abscisic acid-responsive gene expression, and its overexpression affects abscisic acid sensitivity. *Plant Physiol*, **153**(2), 716-27.

Leite, J. P. et al. (2014) Overexpression of the activated form of the *AtAREB1* gene (*AtAREB1DeltaQT*) improves soybean responses to water deficit. *Genet Mol Res*, **13**(3), 6272-86.

Leran, S. et al. (2015) Nitrate sensing and uptake in *Arabidopsis* are enhanced by ABI, a phosphatase inactivated by the stress hormone abscisic acid. *Sci Signal*, **8**(375), ra43.

Liu, Q. et al. (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**(8), 1391-406.

Lu, Y. S. Y. et al. (2015) ABI1 regulates carbon/nitrogen-nutrient signal transduction independent of ABA biosynthesis and canonical ABA signalling pathways in *Arabidopsis*. *J Exp Bot*, **66**(9), 2763-2771.

Marinho, J. P. et al. (2015) Characterization of Molecular and Physiological Responses Under Water Deficit of Genetically Modified Soybean Plants Overexpressing the *AtAREB1* Transcription Factor. *Plant Molecular Biology Reporter*. in press.

Maruyama, K. et al. (2009) Metabolic pathways involved in cold acclimation identified by integrated analysis of metabolites and transcripts regulated by DREB1A and DREB2A. *Plant Physiol*, **150**(4), 1972-80.

Maruyama, K. et al. (2012) Identification of cis-acting promoter elements in cold- and dehydration-induced transcriptional pathways in *Arabidopsis*, rice, and soybean. *DNA Res*, **19**(1), 37-49.

Miyakawa, T. et al. (2012) Structure and function of abscisic acid receptors. *Trends Plant Sci*, **18**, 259-266.

Mizoi, J. et al. (2012) AP2/ERF family transcription factors in plant abiotic stress responses. *Biochim Biophys Acta*, **1819**(2), 86-96.

Nakashima, K. et al. (2009a) Three Arabidopsis SnRK2 protein kinases, SRK2D/SnRK2.2, SRK2E/SnRK2.6/OST1 and SRK2I/SnRK2.3, involved in ABA signaling are essential for the control of seed development and dormancy. *Plant Cell Physiol*, **50**(7), 1345-63.

Nakashima, K. et al. (2009b) Transcriptional regulatory networks in response to abiotic stresses in *Arabidopsis* and grasses. *Plant Physiol*, **149**(1), 88-95.

Nakashima, K. et al. (2013) Comparative functional analysis of six drought-responsive promoters in transgenic rice. *Planta*, **239**, 47-60.

Nakashima, K. et al. (2000) Organization and expression of two *Arabidopsis DREB2* genes encoding DRE-binding proteins involved in dehydration- and high-salinity-responsive gene expression. *Plant Mol Biol*, **42**(4), 657-65.

Nakashima, K. et al. (2012) NAC transcription factors in plant abiotic stress responses. *Biochim Biophys Acta*, **1819**(2), 97-103.

Nakashima, K. et al. (2007) Functional analysis of a NAC-type transcription factor OsNAC6 involved in abiotic and biotic stress-responsive gene expression in rice. *Plant J*, **51**(4), 617-30.

Nakashima, K. and Yamaguchi-Shinozaki, K. (2013) ABA signaling in stress-response and seed development. *Plant Cell Rep*, **32**(7), 959-70.

Nakashima, K. et al. (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.

Narusaka, Y. et al. (2003) Interaction between two cis-acting elements, ABRE and DRE, in ABA-dependent expression of *Arabidopsis rd29A* gene in response to dehydration and high-salinity stresses. *Plant J*, **34**(2), 137-48.

Pandey, S. B. H. (2007) Introduction. *In Economic costs of drought and rice farmers' coping mechanisms*, eds. Pandey, S., Bhandari, H., Hardy, B., 1-9.

Pellegrineschi, A. et al. (2004) Stress-induced expression in wheat of the *Arabidopsis thaliana* DREB1A gene delays water stress symptoms under greenhouse conditions. *Genome*, **47**(3), 493-500.

Pennisi, E. (2008) Plant genetics. The blue revolution, drop by drop, gene by gene. *Science*, **320**(5873), 171-3.

Polizel, A. M. et 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**(4), 3641-56.

Prado, J. R. et al. (2014) Genetically engineered crops: from idea to product. *Annu Rev Plant Biol*, **65**, 769-90.

Raghavendra, A. S. et al. (2010) ABA perception and signalling. *Trends Plant Sci*, **15**(7), 395-401.

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

Redillas, M. C. et al. (2012) The overexpression of *OsNAC9* alters the root architecture of rice plants enhancing drought resistance and grain yield under field conditions. *Plant Biotechnol J*, **10**(7), 792-805.

Reis, R. R. et al. (2014) Induced over-expression of *AtDREB2A CA* improves drought tolerance in sugarcane. *Plant Sci*, **221-222**, 59-68.

Saint Pierre, C. et al. (2012) Phenotyping transgenic wheat for drought resistance. *J Exp Bot*, **63**(5), 1799-808.

Sakuma, Y. et al. (2006a) Functional analysis of an *Arabidopsis* transcription factor, DREB2A, involved in drought-responsive gene expression. *Plant Cell*, **18**(5), 1292-309.

Sakuma, Y. et al. (2006b) Dual function of an *Arabidopsis* transcription factor DREB2A in water-stress-responsive and heat-stress-responsive gene expression. *Proc Natl Acad Sci USA*, **103**(49), 18822-7.

Sato, H. et al. (2014) *Arabidopsis* DPB3-1, a DREB2A interactor, specifically enhances heat stress-induced gene expression by forming a heat stress-specific transcriptional complex with NF-Y subunits. *Plant Cell*, **26**(12), 4954-73.

Shinwari, Z. K. et al. (1998) An *Arabidopsis* gene family encoding DRE/CRT binding proteins involved in low-temperature-responsive gene expression. *Biochem Biophys Res Commun*, **250**(1), 161-70.

Taji, T. et al. (2002) Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana. Plant J*, **29**(4), 417-26.

Takasaki, H. et al. (2010) The abiotic stress-responsive NAC-type transcription factor OsNAC5 regulates stress-inducible genes and stress tolerance in rice. *Mol Genet Genomics*, **284**(3), 173-83.

Takasaki, H. et al. (2015) SNAC-As, stress-responsive NAC transcription factors, mediate ABA-inducible leaf senescence. *Plant J*, in press.

Tran, L. S. et al. (2004) Isolation and functional analysis of *Arabidopsis* stress-inducible NAC transcription factors that bind to a drought-responsive *cis*-element in the early responsive to dehydration stress 1 promoter. *Plant Cell*, **16**(9), 2481-98.

Uga, Y. et al. (2013) Control of root system architecture by *DEEPER ROOTING 1* increases rice yield under drought conditions. *Nat Genet*, **45**(9), 1097-102.

Umezawa, T. et al. (2010) Molecular basis of the core regulatory network in ABA responses: sensing, signaling and transport. *Plant Cell Physiol*, **51**(11), 1821-39.

Xiao, B. et al. (2007) Overexpression of a *LEA* gene in rice improves drought resistance under field conditions. *Theor Appl Genet*, **115**(1), 35-46.

Yadav, S. R. R. et al. (2011) *Crop Adaptation to Climate Change*. Wiley-Blackwell, ISBN-13: 978-0813820163.

Yamaguchi-Shinozaki, K. and Shinozaki, K. (2006) Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu Rev Plant Biol*, **57**, 781-803.

Yoshida, S. and Hasegawa, S. (1982) The rice root system: its development and function. *In Drought resistance in crops with emphasis on rice*, IRRI, Philippines, 97-114.

Yoshida, T. et al. (2015) Four *Arabidopsis* AREB/ABF transcription factors function predominantly in gene expression downstream of SnRK2 kinases in abscisic acid signalling in response to osmotic stress. *Plant Cell Environ*, **38**(1), 35-49.

Yoshida, T. et al. (2010) AREB1, AREB2, and ABF3 are master transcription factors that cooperatively regulate ABRE-dependent ABA signaling involved in drought stress tolerance and require ABA for full activation. *Plant J*, **61**(4), 672-85.

Yoshida, T. et al. (2011) *Arabidopsis* HsfA1 transcription factors function as the main positive regulators in heat shock-responsive gene expression. *Molecular Genetics and Genomics*, **286**(5-6), 321-332.