Chapter 2-1

Outline of the Project Entitled "Development of Drought-Tolerant Crops for Developing Countries"

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Abstract

Global warming is expected to have serious effects on nature and society. The agricultural, forestry, and fishing industries have faced challenges with the increasing frequency of abnormal climate events, such as flooding and drought. These climate changes, along with soil degradation, have caused damages to crop production. In the light of these situations, in 2013, Japan International Research Center for Agricultural Sciences (JIRCAS) launched a research project on development of drought-tolerant rice and wheat for developing countries in collaboration with the International Rice Research Institute (IRRI), the International Center for Tropical Agriculture (CIAT), and the International Maize and Wheat Improvement Center (CIMMYT). We have identified 14 genes involved in the drought tolerance and three stress-inducible promoters. These genes were introduced into lowland rice variety IR64, upland rice variety Curinga and NERICA, wheat variety Fielder. In addition, in order to remove unexpected DNA fragments inserted during transformation or cultivation, we carried out a cleaning of genetic background by backcrossing with the parental variety. We also addressed to pile up drought tolerance genes or QTLs, to confer more tolerance to the promising lines. Based on physiological traits and grain yield under drought stress conditions in a greenhouse and confined field, we have so far narrowed-down following promising lines of IR64, Curinga, NERICA, and Fielder.

Outline of the project

Global food demand is expected to rise due to expanding world population in Southeast Asia and Africa, as well as chronic nutrient deficiency and rapid economic growth in emerging countries, China and India. On the other hand, food supply will decrease, with frequent abnormal weather conditions, such as water resources shortage, and soil degradation including desertification. In the medium and long term, the global food supply-demand balance will be tight, and food production will require an increase of 160% by 2050. However, we are now faced with various environmental risks, including soil and water pollution, deforestation, and soil degradation, as well as climate change risks, such as drought, high temperature, and flooding. Climate change is expected to have serious effects on nature and society around the world. The increasing threat of climate change is manifested by extreme weather events such as high temperature, drought and heavy rainfall, which are becoming more frequent. Although climate change has had a positive effect on crop production in temperate regions. On the other hand, in tropical and subtropical regions including South-East Asia and Africa, the crop production is estimated to be reduced by 5% to 50%, even though there is a need for increase in food production (**Fig. 1**).

In the field of agriculture, forestry, and fisheries, it is forecasted to pose detrimental effects on the production of agricultural products in the world, particularly in developing countries, which are the most vulnerable to climate change.

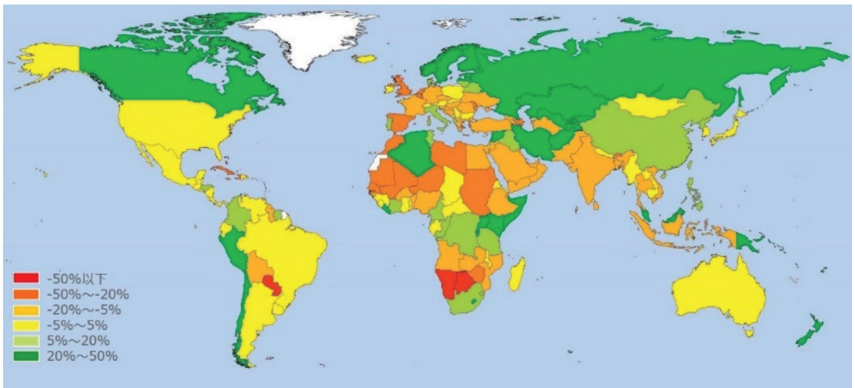

Fig. 1. Potential Effects of Climate Change on Rain-fed Cereal Production (1990 – 2050).
The data are cited from Global Agro-ecological Assessment for Agriculture in the 21st Century: Methodology and Results, FAO, 2002.

In the light of these situations, in 2013, the Ministry of Agriculture, Forestry and Fisheries in Japan established a research program on the development of adaptation and mitigation technology in response to climate change, in order to achieve a sustainable food production system that could adapt to the progressing global warming. Under this program, JIRCAS implemented a research project on the development of drought-tolerant crops for developing countries (GM Drought Tolerance Project), in collaboration with member institutes of the Consultative Group on International Agricultural Research (CGIAR).

We had previously implemented a research project on development of abiotic stress tolerant crops by DREB gene (DREB Project) in 2008. In this project, we had identified crucial genes involved in drought stress tolerance, including *Arabidopsis* transcription factor DREB1; rice putative RNA binding protein OsSCZF2 (TZF5); *Arabidopsis* AtGolS2, which is a key enzyme in galactinol biosynthesis; *Arabidopsis* ABA responsive transcription factor AREB. We had also isolated three stress inducible promoters, such as rice *Oshox 24*, *Osnac 6*, and *lip 9* promoter. Then, we introduced these genes into lowland rice variety IR64, upland rice variety Curinga and NERICA, wheat variety Fielder. Based on physiological traits and grain yield under drought stress conditions in a greenhouse and confined field, we had selected 7 events of IR64, 16 events of Curinga, 4 events of NERICA, and 22 events of Fielder (Nakashima and Suenaga 2017).

With the help of the results obtained from previous study, we launched a research project 5 years ago in 2013. This project is aimed at developing 10 drought tolerant rice and wheat lines for developing countries, in collaboration with IRRI, CIAT, and CIMMYT. IRRI, CIAT, and CIMMYT mainly conducted field evaluation of the promising lines of lowland rice, upland rice, NERICA, and wheat, and selected 2 or 3 elite lines from each variety. On the other hand, JIRCAS carried out molecular evaluation of the promising lines selected by IRRI, CIAT, and CIMMYT (**Fig. 2**).

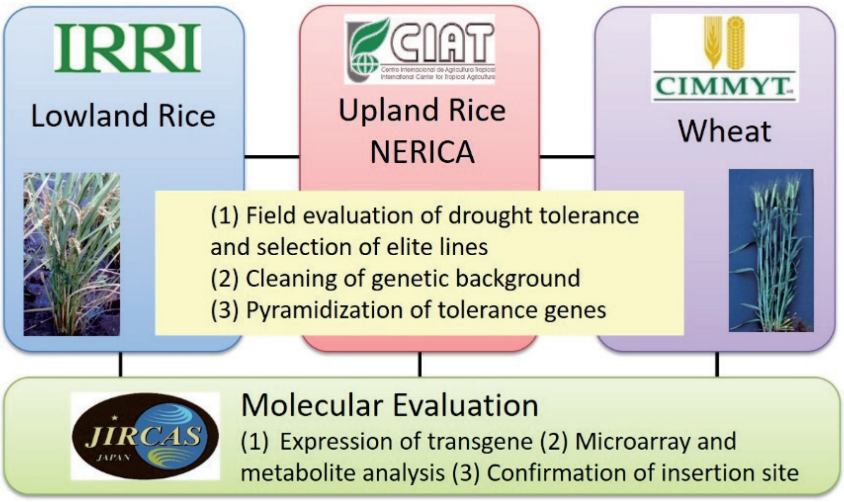

Fig. 2. Framework of GM Drought Tolerance Project.

IRRI, CIAT, and CIMMYT had 3 major research activities: (1) verification of drought tolerance by confined field trials, (2) cleaning of genetic background of the promising lines, (3) pyramiding of tolerance genes (QTLs). Each institute carried out confined large-scale field trials and investigated grain yield. They also evaluated physiological traits such as stomata conductance, harvest index, and water uptake rate, as criteria for drought tolerance. The promising lines were further evaluated in the field to verify the effects of the transgenes so that we could narrow down the number of promising lines, leaving only elite lines. A series of 3 to 4 confined field trials were performed with plot sizes larger than those in the DREB Project (**Fig. 3**).

Fig. 3. Field evaluation of drought tolerance and selection of elite lines.

The 2nd activity was cleaning genetic background. Fragments of transgenes may be introduced upon transformation and remain in promising lines, which would have potentially interfered with future safety assessments. Therefore, genetic background had to be replaced to create adequate breeding material. We conducted a minimum of 3 backcross breedings, using commercial varieties, IR64, Curinga, NERICA, and Reedling as recurrent parents. Consequently, undesirable DNA fragments derived from the transformation vectors, which were at risk for being incorporated by transformation, were removed. The 3rd activity was stacking of genes for drought tolerance in candidate elite lines to confer higher drought tolerance. Major activities of JIRCAS were (1) analysis of expression of the transgenes in the promising and backcrossed lines, (2) transcriptome and metabolome analysis, (3) confirmation of flanking sequences of the insertion site in transgenic lines. JIRCAS also evaluated agronomic traits of transgenic NERICA lines in a greenhouse setting. Based on physiological traits and grain yield under drought stress conditions in a greenhouse and confined field, we were able to narrow the crop selection to 3–4 promising lines of IR64, Curinga, NERICA, and Fielder.

Recently we have succeeded in developing transgenic rice lines that overexpress *AtGolS2*, which is a candidate gene for drought tolerance encoding a galactinol synthase identified in *Arabidopsis* and presented increased grain yield in transgenic rice under drought in the field (Selvaraj et al. 2017). We generated transgenic rice lines that express *AtGolS2* in two varieties, Curinga and NERICA4. Curinga is a Brazilian local upland rice variety, and NERICA4 is a popular upland rice variety in African countries. Each transgenic line accumulated significantly higher amounts of galactinol as compared to that in non-transgenic rice plant (**Fig. 4**). The transgenic lines grown under drought had higher relative water content in leaves and higher photosynthetic activity than non-transgenic plants, leading to lesser reduction in plant growth. In order to test the performance of the transgenic lines under drought in the field, three consecutive field trials were carried out. The extent of drought varied among trial years. For instance, trial years 2012–2013 and 2013–2014 were very dry with continuous rain-free days (31 days and 39 days, respectively), including flowering periods. However, there were only 19 rain-free days after flowering in trial year 2014–2015. A transgenic Curinga line (numbered 2580) and a transgenic NERICA4 line (numbered 1577) consistently had higher grain yield than each non-transgenic variety (**Fig. 5**). These results provide a strong evidence that *AtGolS2* is a useful biotechnological tool to reduce grain yield losses in rice under drought in the field.

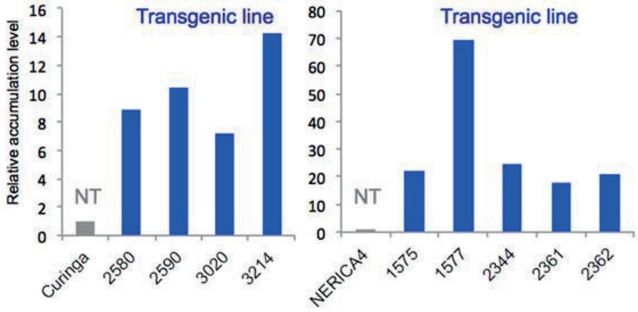

Fig. 4. Accumulation of galactinol in transgenic lines for *AtGolS2*.
Numbers indicate the identification number used for each transgenic line. NT indicates non-transgenic plants.

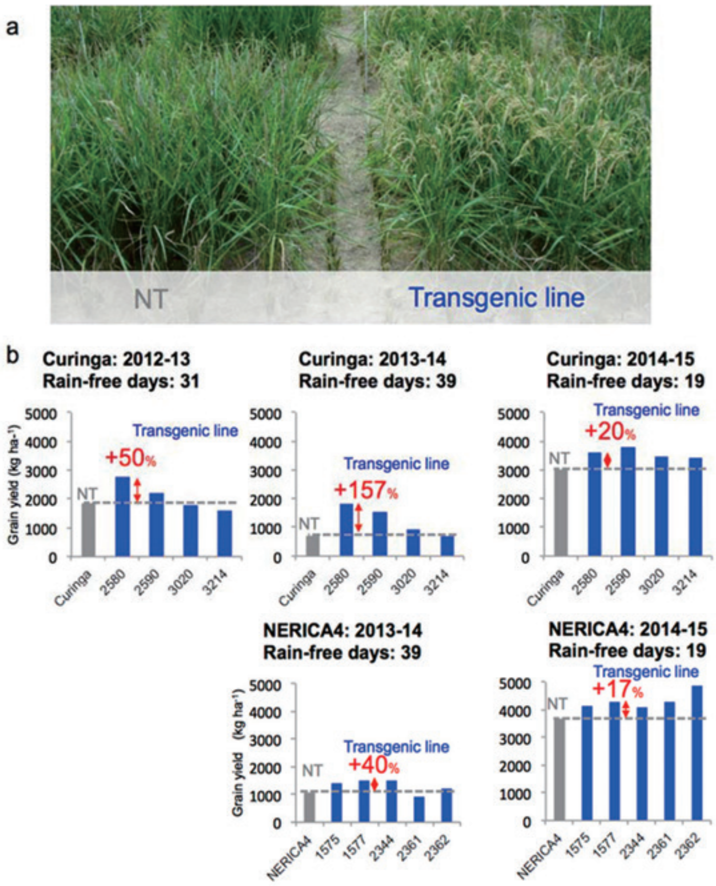

Fig. 5. Improved grain yields of transgenic lines for *AtGolS2* under drought in the field.
(a) Evaluation of transgenic rice in a confined field in CIAT. Left, non-transgenic Curinga; right, transgenic lines of Curinga (numbered 2580). (b) Grain yield of transgenic lines for *AtGolS2* in the three consecutive field trials. Numbers indicate the identification number used for each transgenic line. NT indicates non-transgenic plants.

In the final year of the project, we held an international workshop at Tsukuba, Japan. In this workshop, the research results of the past five years and current situation and future prospects for development of GM crops were presented, and we discussed issues to be addressed for practical application of GM crops and the possibility of collaboration (**Fig. 6**). In the first session of the workshop, IRRI, CIAT, CIMMYT, and JIRCAS, reported the research results obtained over the past five years. In the second session, the invited speakers presented the status of current research and development of GM crops and their dissemination in Africa. Among the initiatives pursued was the NEWEST (Nitrogen-Use Efficient, Water-Use Efficient and Salt-Tolerant) rice program. Based on its results to date, this program could serve as a good model for practical application of GM crops. Finally, in the general discussion session, participants exchanged ideas and viewpoints on issues that need to be addressed in order to realize the practical application of GM crops in Africa. We also discussed the prospect for cooperation between JIRCAS and relevant African organizations, as well as the mutual concerns shared by both parties. We recognize the need to exert further efforts to disseminate research outputs in developing countries, in collaboration with farmers, private companies, and extension organizations. We hope that the discussion in this workshop will become the first step toward the promotion of social implementation in our research outputs.

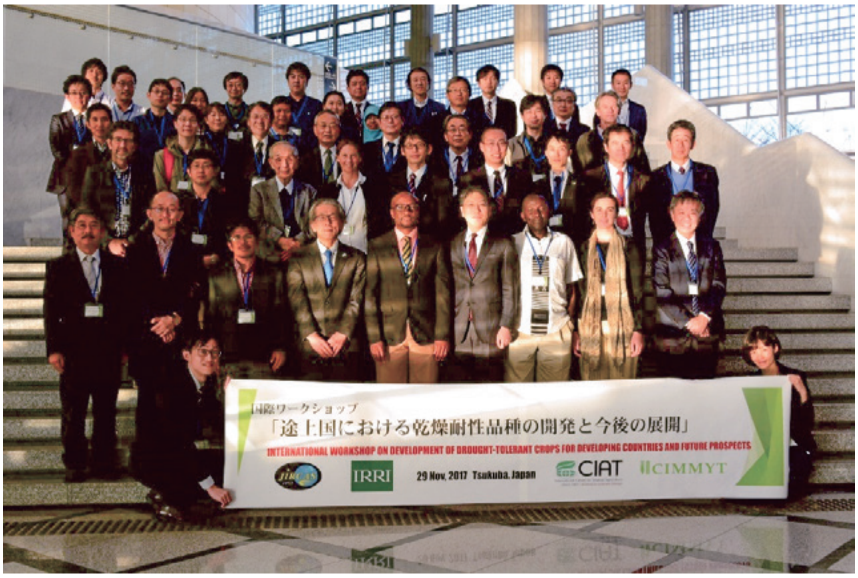

Fig. 6. All speakers, chairs, and participants in the workshop.

While preparing this manuscript, a paper titled "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" was published (Selvaraj et al. 2020).

References

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