

JIRCAS Newsletter

for
INTERNATIONAL COLLABORATION



Evaluation of drought-tolerance of soybean in Brazil (Photo by Embrapa)

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Toward Stable Crop Production under Various Stresses

The Intergovernmental Panel on Climate Change (IPCC), which held its 38th general assembly in Yokohama in March, has declared global warming as an ongoing threat to food security due to the adverse effects of extreme weather events such as floods and droughts. According to its September 2013 evaluation report, global average temperature had increased by 0.85°C during the past 130 years (up until 2012) and is projected to rise — between 0.3 to 4.8°C — by 2100. Temperature increase since the mid-20th century has been attributed to the accumulation of greenhouse gases (mainly carbon dioxide) in the atmosphere caused by anthropological or human activities. Due to global warming, average annual rainfall is expected to increase in mid-high latitude regions and decrease in the arid subtropical areas.

News of extreme weather events such as floods and droughts are now heard more often than before. Each year, many parts of the world report various damages to crops due to droughts, resulting to declining harvests. This has implications in Japan as well. Japan, a heavy noodle consumer, relies on Australian wheat, which was affected by droughts in 2006-2007. Russia, another top exporter, imposed a grain embargo on wheat in 2010 when a severe drought struck the country, resulting to a huge impact on global supply. Last year's droughts in South America and the United States are still fresh in memory.

Factors that threaten the stability of crop production include stresses, of which there are two types: biotic stresses, such as diseases and pests; and abiotic stresses, such as droughts and salt damage. Besides drought, salt damage has also been causing major problems worldwide. It has affected about 77 million hectares of arable land (mainly in arid/semi-arid regions and coastal areas) and 45 million hectares (20% of total) of irrigated agricultural land (Tanji, 2002). This problem is expected to linger into the future. Another abiotic stress that needs to be remedied is phosphorus deficiency in soils. Meanwhile, biotic stresses such as pests and diseases in crops are becoming widespread around the world, adversely affecting developing countries in particular. For example, soybean rust disease has been inflicting severe damage in South America since 2001, and is costing Brazil approximately \$2 billion a year just for disease control. Another example is rice blast, a serious fungal disease affecting temperate to tropical rice.

JIRCAS, in cooperation with research institutes in developing countries, has been carrying out research and development of agriculture, forestry and fisheries technology with the aim of solving global food and environmental issues. Its activities include combatting various stresses that threaten the stable production of crops

in developing regions.

One approach being adopted for stable crop production in the developing world is through genetic and breeding technologies. First, wide ranges of genetic resources are evaluated, and selected germplasms with disease resistance or drought tolerance are analyzed. Afterwards, marker development as well as identification and isolation of genes are done to acquire the basic techniques and materials for developing strong varieties that could overcome various stresses.

Drought is a very serious problem, thus molecular biological research over the past 20-plus years has focused on identifying, isolating, and elucidating the function of various tolerance genes. Likewise, identification of resistance genes, functional analysis, and marker development are being performed in response to soil salinity and phosphate deficiency. The identified and isolated genes will be introduced into a variety of crops to help develop high-level tolerant crops. Resistant varieties capable of combatting crop diseases are also being developed. Data collection and analysis have generated plenty of information on pathogenic variations, and a number of useful genes identified to date are being introduced into soybean, rice and other crops.

The development of tolerant/resistant varieties — from the selection of genetic resources and identification of genes to the development of varieties — takes a considerable amount of time, including the period taken by development of materials and fundamental technologies. A long-term perspective is therefore required to sustain the research and development activity. For Japan, which relies heavily on food imports (with the exception of rice), ensuring the stability of crop production in major exporting countries is crucial because it seriously affects its own food supply and demand stability.



Drought withers plants in Australia. (Photo credit: NHK Creative Library)

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International Research Network for Rice Blast Disease

Blast studies in the world

Rice blast (*Pyricularia oryzae* Cava) is one of the most serious rice diseases in the world. A number of studies were carried out, and advanced technologies and knowledge related to the development of a protection system against blast disease have been accumulated in Japan, including (1) the development of a differential system to clarify the pathogenicity of blast isolates, (2) the genotyping of resistance genes in rice varieties, (3) the application of novel resistance genes in breeding, (4) the development of near-isogenic lines (NILs) for blast resistance genes in Japonica-type rice cultivars, Koshihikari and Sasanishiki, and (5) the creation of a multiline variety from NILs with the genetic background of Koshihikari.

Serious damages were found in the upland and rainfed lowland fields at high elevations and at monoculture fields in the irrigated lowlands of tropical regions. However, systematic studies were not done in the developing countries of tropical regions. Because the farmers and scientists failed to recognize the damages caused by blast fungus infection, evaluating the degree of infection and managing the blast fungus proved more difficult compared with other rice diseases.

The most cost-effective technology and method against blast disease is the use of a resistant variety harboring effective gene(s) against dominant blast races. To develop the effective resistant variety, the differential system is needed. This consists of (1) a differential varieties' set which has different resistance genes in each genetic background and (2) a standard differential blast isolates' set which clarifies the pathogenicity. Unfortunately, the differential system has yet to be developed and used in developing countries in tropical regions because the differential varieties for blast fungus were not useful.

Collaboration with the International Rice Research Institute (IRRI)

The Japan International Research for Agricultural Sciences (JIRCAS), together with IRRI, developed an international differential varieties' set for blast fungus. These differential varieties were developed by backcross breeding with a susceptible Chinese variety, Lijiangxintuanheigu (LTH), with each variety introduced

with a different resistance gene in the genetic background. To date, the number of resistance genes used for differential varieties, which were developed and selected from previous studies, is the biggest and are the most useful materials among these sets.

International network studies using the differential varieties' set

In cooperation with national research institutes and universities in Southeast and South Asia, an international collaborative research on blast disease is being conducted using the international differential varieties developed by JIRCAS and IRRI. These studies aim to create a durable protection system against blast disease through the development and application of the differential system (Fig. 1).

The network studies used (1) a common criteria for evaluating the virulence of blast isolates to each differential variety and (2) a designation method for blast isolates based on the reaction patterns to differential varieties' set as race (Fig. 2), allowing all data to be shared and compared among the participants. As a result, (3) the diversity and distribution of blast races at regional and global levels were clarified, and (4) the use of international standard differential blast isolates (ISDBIs) made possible the characterization of resistance genes and the evaluation of resistance in rice varieties. In the breeding studies, (5) the genetic variation of resistance in rice germplasm, (6) the clarification of genotypes of resistance genes of leading varieties in each country, and (7) the development of multiline varieties with Indica-type genetic backgrounds which will be used as materials under a new protection system using genetic diversity, were carried out.

These studies on blast disease using the differential system have been adopted by other research networks such as IRRI's Temperate Rice Research Consortium (TRRC), which focuses on temperate rice agriculture, and the Africa Rice Center (AfricaRice), whose research project targets rice diseases in West and East Africa. JIRCAS, via research networks, will contribute to the continuous improvement of rice agriculture in developing countries in Asia and Africa through development and delivery of a durable protection system for blast disease.

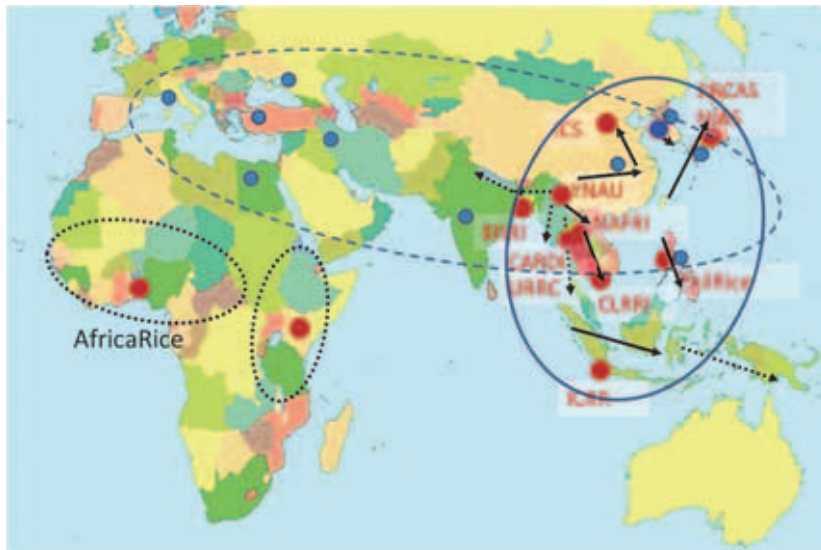


Fig. 1. Three international research networks for studying rice diseases in the world

- ✧ JIRCAS is conducting a research project, titled “Blast Research Network for Stable Rice Production,” in the tropical regions of Asia.
- ✧ The International Rice Research Institute (IRRI) has organized a research collaborative group, the Temperate Rice Research Consortium (TRRC), and blast study is one of its research subjects.
- ✧ Africa Rice Center (AfricaRice) is conducting research on rice diseases in West and East Africa.
- ✧ These research projects by IRRI and AfricaRice adopt the same system and methods developed by JIRCAS.



Fig. 2. Standard evaluation for pathogenicity and designation methods for blast race

- ✧ Pathogenicity of blast isolate is investigated based on the reaction of 25 differential varieties and a susceptible variety, LTH, and categorized into 6 scores (Resistance 0-5: Susceptible) (Fig. 2, left).
- ✧ The ranges of reaction scores for virulence of blast isolates vary among 25 differential varieties for targeting 23 resistance genes, and the final evaluation for comparability of blast isolate is decided based on the panel (Fig. 2, left).
- ✧ Designation of race is decided based on the reaction patterns of final evaluations to 25 differential varieties and LTH (Fig. 2, right)
- ✧ These standard evaluation and designation methods have been translated into 12 languages.

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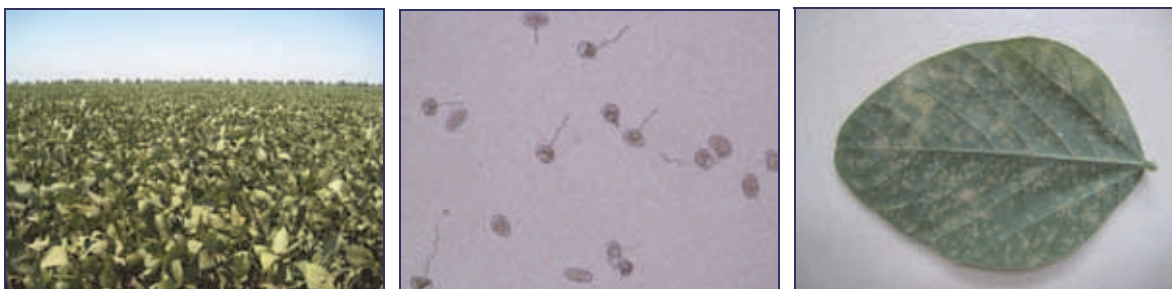
Analysis of Pathogenic Variation of Soybean Rust in South America

Soybean rust, caused by the plant pathogenic basidiomycete fungus *Phakopsora pachyrhizi*, was first recorded in Japan in 1902. It was not found in South America until 2001, when it was first described in Paraguay. Since then, it has spread to Brazil, Argentina, Uruguay, and Bolivia, becoming one of the most destructive and economically important diseases of soybean in South America. According to the Food and Agriculture Organization of the United Nations, South American countries are the largest soybean producers in the world, with Brazil, Argentina, and Paraguay accounting for approximately half of world soybean production and 54% of global exports. Prior to pursuing actual strategies to combat soybean rust in South America, understanding the pathogenicity of indigenous fungal populations would be useful for identifying resistant plant genotypes and for targeting effective cultivars against certain populations. A JIRCAS project was launched in cooperation with research institutes from Argentina, Brazil, and Paraguay to investigate and compare the rust pathogenicity of *P. pachyrhizi* infecting soybean in the three South American countries in 2007–2010.

Soybean rust samples were collected in Argentina, Brazil, and Paraguay covering three cropping seasons (2007/2008, 2008/2009, and 2009/2010) while rust samples were collected in Japan in 2007 and 2008 for comparison. Sixteen soybean genotypes including cultivars and lines were selected as a differential set to test soybean rust populations from South American countries. These were grown in a growth chamber at 24°C, subjected to a 14 h photoperiod under rust-free conditions, and inoculated with *P. pachyrhizi* urediniospore suspension using a paintbrush or a glass atomizer. Two weeks after inoculation, lesion appearance [presence (+) or absence (-) of lesions], sporulation level (SL), and the number of uredinia per lesion (NoU) on the differential set, were determined. Data for the three parameters -- (i) lesion appearance, (ii) SL, and (iii) NoU -- were collected for all rust populations and converted into infection types caused by the rust populations: ‘susceptible’ indicated in blue, ‘intermediate’

in orange, and ‘resistant’ in red. As the number of susceptible infection type increases in a rust sample, the sample shows more virulence to the 16 differentials.

Using the 16 soybean differentials, 61 rust samples (30 of which are shown in the table) were collected from Argentina, Brazil, and Paraguay in three cropping seasons of 2007–2010 to evaluate for pathogenicity. Among South American populations that were analyzed, only two pairs yielded identical pathogenicity profiles in the 16 differentials: BSE4-2 and PMA5-3 from Brazil and Paraguay, respectively, and PNC1-1 and PMA9-1 from Paraguay, indicating substantial pathogenic variation in the rust populations. Each of the rust samples with identical pathogenicity profiles was collected from different locations, suggesting no association between pathogenicity and geographical origins of the samples. Comparative analysis of 61 South American and five Japanese samples revealed that pathogenic differences not only were detected within South America but were also distinct between the *P. pachyrhizi* populations from South America and Japan. In addition, pathogenic differences were observed among South American *P. pachyrhizi* populations with the same geographical origin but different temporal origins. Thus, yearly changes in rust pathogenicity were detected during the sampling period. The differentials containing resistance genes *Rpp1* (except for PI 587880A, PI 587905, and PI 594767A), *Rpp2*, *Rpp3*, and *Rpp4*, displayed resistant reaction to only 1.8%–14%, 24%–28%, 22%–31%, and 36% of South American *P. pachyrhizi* populations, respectively. In contrast, three *Rpp1*-carrying differentials (PI 587880A, PI 587905, and PI 594767A), the *Rpp5*-carrying differential (Shiranui), and one *Rpp*-unknown differential (PI 587855) showed resistant reaction to 78%–96% of all populations. This study demonstrated pathogenic diversity of *P. pachyrhizi* populations in South America, and that the known *Rpp* genes other than *Rpp1* (in PI 587880A, PI 587905, and PI 594767A) and *Rpp5* have been less effective against recent pathogen populations in the countries studied.



Photos: Soybean field in Paraguay, South America (left), urediniospores of soybean rust (middle), and soybean rust-diseased leaflet (right)

Table: Infection types of *Phakopsora pachyrhizi* samples from South America collected in 2007–2010 on the 16 differentials ^a

	<i>Phakopsora pachyrhizi</i> sample ^b	Cropping season	Differential															
			1. PI 200492 (<i>Rpp1</i>)	2. PI 368039 (<i>Rpp1</i>)	3. PI 230970 (<i>Rpp2</i>)	4. PI 417125 (<i>Rpp2</i>)	5. PI 462312 (<i>Rpp3</i>)	6. PI 459025 (<i>Rpp4</i>)	7. Shiranui (<i>Rpp5</i>)	8. PI 416764 (<i>Rpp3</i>)	9. PI 587855	10. PI 587880A (<i>Rpp1</i>)	11. PI 587886 (<i>Rpp1</i>)	12. PI 587905 (<i>Rpp1</i>)	13. PI 594767A (<i>Rpp1</i>)	14. BRS 154	15. TK5	16. Wayne
Argentina	APM1-1		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	APM1-3		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	APM2-2		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	APM2-3		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	ANE6-2		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	ANE6-3		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	ANE7-1		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	ANE7-3		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	ANW10-3		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	ANW12-2		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
Brazil	BSO1-1		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	BSO1-2		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	BSO1-3		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	BSE4-2		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	BCW11-1		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	BCW11-2		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	BCW11-3		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	BNO12-1		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	BNO12-3		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	BNE14-2		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
Paraguay	PNC1-1		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	PMA5-3		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	PMA6-1		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	PMA6-2		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	PMA9-1		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	PSI13-1		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	PSI13-2		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	PSI15-1		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	PSI15-2		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	PSI15-3		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
Japan	JRP		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	T1-2		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	N1-1		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	E1-4		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	N2-1		Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue

^a Classification of infection types: susceptible indicated in blue ■; intermediate in orange ■; resistant in red ■. The column with grey ■ indicates that infection type was not determined because of preparation failure.

^b APM: Pampa region; ANE: Northeast region; ANW: Northwest region; BSO: South region; BSE: Southeast region; BCW: Central-West region; BNO: North region; BNE: Northeast region; PNC: Canindeyú prefecture; PMA: Alto Paraná prefecture; PSI: Itapúa prefecture. The following numbers represent sampling locations in each country (1–12, 1–14, and 1–15) and sampling season (1: 2007/08 ■; 2: 2008/09 ■; 3: 2009/10 ■). Samples with the same location number were derived from the same location. Samples derived from the same location were indicated in yellow ■ in each location.

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Soybean Breeding for Asian Soybean Rust Resistance in South America

Imports account for most of Japan's domestic soybean consumption; therefore, it is very important that soybean production in South America, which supplies most of the soybean in the world market, remains stable. Development of soybean cultivars that show durable resistance to Asian soybean rust (ASR), a major soybean disease, will help reduce its huge control costs, which in turn will result to improved soybean productivity and farmers' profits. However, as mentioned in the previous report, titled "Analysis of Pathogenic Variation of Soybean Rust in South America," the pathogenicity of ASR pathogens in South America is diverse and strong. Thus, we may not expect stable and durable resistance in the resistant varieties, which were developed by introducing one of six known ASR resistance genes (*Rpp1* - *Rpp6*). In order to cope with the peculiar characteristics of ASR pathogens in South America, four schemes were considered, namely, 1) using new ASR resistance genes in soybean; 2) pyramiding known ASR resistance genes; 3) using field resistance or tolerance against ASR; or 4) using disease-resistant genes isolated from other species. We then carried out research studies on each of these four schemes for developing new ASR resistant cultivars in South America.

From the results of our studies to date, pyramiding known ASR resistance genes (scheme 2) is considered the most appropriate due to its effect on resistance and its convenience in breeding programs; thus, we developed some soybean materials to be used for the breeding programs in South America. Specifically, we developed soybean lines carrying multiple known ASR resistance genes--the so-called '*Rpp*-pyramided lines'--using known soybean varieties (each carrying a single ASR resistance gene) and DNA markers (to check if the plants have ASR resistance genes or not). We then evaluated the resistance of these *Rpp*-pyramided lines against some ASR pathogens having especially high virulence among South American ASR pathogens, and observed much higher resistance in some *Rpp*-pyramided lines compared to their original varieties which carried single ASR resistance genes. In other words, introducing multiple ASR resistance genes together into cultivars should confer not only resistance to a larger number of ASR pathogens but also higher resistance.

We are currently carrying out soybean breeding programs for ASR resistance with 3 institutions: at Nikkei-Cetapar and the Instituto Paraguayo de Tecnología Agraria (IPTA) in Paraguay, and the Instituto Nacional de Tecnología Agropecuaria (INTA) in Argentina using the line which showed the highest resistance among our *Rpp*-

pyramided lines. Specifically, we are developing elite soybean cultivars with both characteristics: high ASR resistance from the donor (*Rpp*-pyramided line developed by JIRCAS), and superior agricultural traits from the recurrent parents (the South American cultivars developed by these three institutions) by recurrent backcrossing or line breeding. DNA markers for tagging ASR genes are also being utilized for these breeding programs. Elite soybean cultivars possessing high resistance against ASR are expected to contribute in reducing ASR control costs and in improving farmers' profits in these countries.

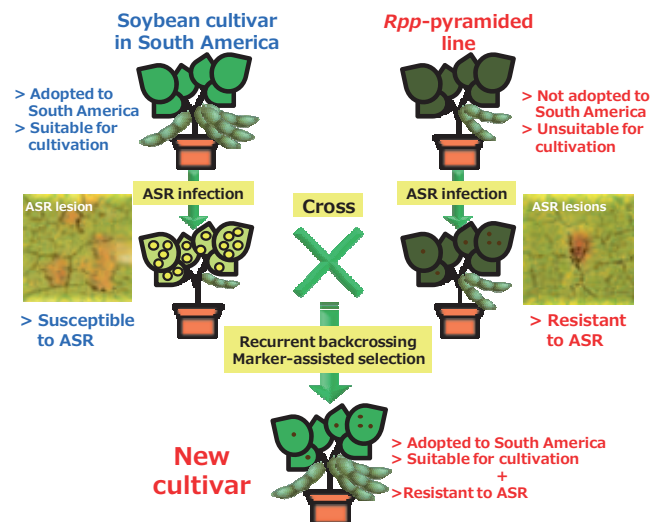


Figure: Scheme of soybean breeding program for Asian soybean rust (ASR) resistance in South America



Photo: Recurrent backcrossing performed in Paraguay

Naoki Yamanaka

Biological Resources and Post-harvest Division

International Joint Research for the Development of Genetically Modified Crops Tolerant to Drought

In recent years, extreme weather events have become more frequent all around the world. Droughts, in particular, have caused food production to decline significantly. The 2012 US drought devastated corn and soybean production, and had a tremendous impact on the world food supply. In Brazil, the drought that occurred in 2012/2013 and 2013/2014 cropping years likewise caused damage to soybean production. Developing drought-tolerant crops, therefore, is required in order to reduce the risk of severe damage to crop production (especially rice, wheat, and other major crops).

Using rice and Arabidopsis as model experimental plants, we studied the molecular mechanisms in environmental stress response for more than 20 years, and our studies revealed that overexpression of stress-responsive transcription factors such as DREB, AREB, and NAC enhanced stress tolerance in laboratory and greenhouse experiments. We are currently working on a joint research project with overseas research institutes to examine whether these genes enhance stress tolerance in other crops or improve stress tolerance under field conditions. This article provides an overview and describes the current status of the international joint research.

[Study on rice and wheat]

In collaboration with the International Rice Research Institute (IRRI) in the Philippines, the International Tropical Agriculture Center (CIAT) in Colombia, and the International Maize & Wheat Improvement Center (CIMMYT) in Mexico, we introduced genes such as *DREB1* into lowland rice, upland rice, and wheat to evaluate drought tolerance in field environments. The research project, titled "Development of abiotic stress tolerant crops by DREB genes" (a.k.a. DREB Project), was supported by the Ministry of Agriculture, Forestry and Fisheries (MAFF) from 2007 to 2013. During the project, RIKEN and JIRCAS made 32 combinations (constructs) of five promoters and 14 tolerance genes, and distributed them to IRRI, CIAT and CIMMYT. More than 1,100 independent transformation events were produced from about 350,000 callus or embryos. Drought tolerance tests using these events were conducted to evaluate grain yield in greenhouses, rainout shelters, and confined fields, and about 40 elite candidate events were selected. A new project, named "Development of drought-tolerant crops for developing countries," was started from 2014, taking over the results from the previous project. This ensuing project aims to develop at least 10 elite events in three countries using the elite candidates selected from the DREB Project.

[Study on soybean]

In collaboration with Embrapa Soybean in Brazil, we introduced *DREB1* genes into soybean and evaluated drought tolerance in greenhouses and confined fields. The Science and Technology Research Partnership for

Sustainable Development (SATREPS), a Japan Science and Technology Agency (JST) and Japan International Cooperation Agency (JICA) program, has been supporting this project since 2009. Soybeans expressing *DREB* or *AREB* genes were generated to show improved tolerance in greenhouses, and field trials are being conducted. In the case of soybean, it is difficult to produce transformants because transformation efficiency is very low. Nevertheless, we succeeded in improving the transformation efficiency of soybean using *Agrobacterium*, hence we can expect transgenic materials to be produced more efficiently in the future. Given the current situation in which genetically modified (GM) soybean is cultivated in 80% of the soybean production areas of the world, a GM soybean variety showing improved drought tolerance can be grown practically anywhere in the world.

[Studies on other crops]

In collaboration with overseas research institutes, we introduced the *DREB* genes into other crops. The International Semi-arid Tropical Areas Crop Research Institute (ICRISAT) in India showed a successful test case for the *DREB1* transgenic peanuts. They reported that the peanuts expressing *DREB1* showed drought tolerance in greenhouse conditions, and that its drought tolerance was also confirmed in field trials. Tests on transgenic sugarcane also gave good results in Embrapa Agroenergy, and further research is expected.

In collaboration with international institutions, we showed that some genes with enhanced drought tolerance in greenhouses and in laboratory tests also displayed improved drought tolerance in field trials. This means that such genes could work under different genetic backgrounds, growth stages, and environmental conditions. We now have plans involving gene transfer as well as field trials of many crop varieties. We hope that the crops being developed through our international joint research contribute toward stabilizing agricultural production and ensuring sufficient food supply worldwide.

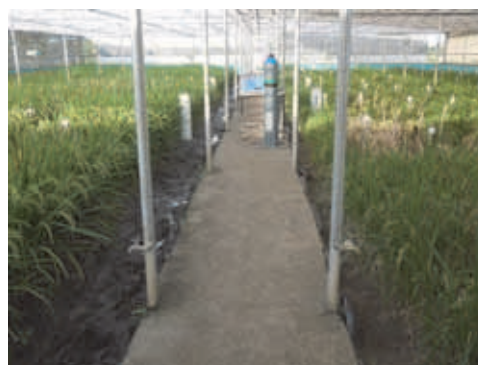


Photo: Drought tolerance test at IRRI

Kazuo Nakashima
Biological Resources and Post-harvest Division

Abscisic Acid and Stress Tolerance in Plants

About 70% of crop yield losses are reported to have been caused by abiotic stresses such as drought, high salinity, heat, and cold/freezing, making it the biggest limitation to global crop production. Worldwide climate change, meanwhile, has increased the frequency and severity of damages due to various abiotic stresses, especially drought and heat, causing serious yield losses in major crops such as maize, wheat and soybean. Given the economic damage to crops during the last several decades, drought is undoubtedly one of the greatest threats to the stability of agricultural production. More than 50% of the world's land, including a large portion of arable land, is regarded as vulnerable to drought.

The plant hormone abscisic acid (ABA) plays a crucial role in the regulation of abiotic stress tolerance in plants (Figure). It controls ABA signaling pathways involved in response to reduced water availability as well as in multiple developmental processes such as seed dormancy. Seed maturation and abiotic stresses such as drought, cold, and high salinity during vegetative stage cause cellular dehydration, which in turn increases endogenous ABA levels in plants through increased ABA biosynthesis in vascular tissues, transports ABA action to sites remote from the sites of biosynthesis, and adjusts ABA metabolism. ABA-bound pyrabactin resistance1/PYR1-like/regulatory components of ABA receptor (PYR/PYL/RCAR) ABA receptor proteins potentiate the formation of protein phosphatases 2C (PP2C)–PYR/PYL/RCAR–ABA ternary complexes to impede the activity of group-A PP2Cs, thereby enabling activation of subclass III sucrose non-fermenting-1 related protein kinase 2 (SnRK2) protein kinases. Three subclass III SnRK2 protein kinases (SRK2D/SnRK2.2, SRK2E/SnRK2.6/OST1 and SRK2I/SnRK2.3) phosphorylate and positively control ABA-mediated transcriptional regulation through the activation of basic leucine zipper (bZIP) transcription factors, including ABA-responsive element binding protein /ABRE-binding factor (AREB/ABFs) and ABA insensitive5 (ABI5) in vegetative tissues and seeds, respectively. The activated SnRK2s also facilitate ABA-induced stomatal closure by phosphorylating anion channels such as slow anion channel-associated1 (SLAC1).

Interestingly, unlike the other phytohormone signaling pathways as seen in ethylene and auxin, the PYR/PYL/RCAR–PP2C–SnRK2-mediated core ABA signaling pathway revealed in model plant *Arabidopsis* is well conserved only in land plants. According to fossil records, the plants first colonized the land about 500 million years ago. Together with previous observations on the role of ABA signaling pathway involved in cellular dehydration in plants, these findings support the notion that core ABA signaling pathway may have played a crucial role in plant colonization of land. Genomic analyses combined with functional data have shown that PYR/

PYL/RCARs, SnRK2s, and AREB/ABF/ABI5-type bZIP transcription factors appeared during plant colonization of land. In addition, transcriptome analyses integrated with phylogenetic data provide the hypothesis that plants have evolved a sophisticated mechanism to cope with environmental stresses via the expansion of duplicate gene families in ABA signaling pathways. Thus, these observations suggest that the emergence of core ABA signaling components has contributed to creating a plant-specific network to adapt to water limitation when plants colonized land. Therefore, elucidation of the mechanism in which plants have adapted to past drastic global environmental changes is considered an important clue to developing stress-tolerant crops that can cope with future global climate change.

Many genes involved in stress response and tolerance have been identified as the mechanisms of response and tolerance in plants have been revealed. To date, transcription factors such as AREB, which were identified relatively earlier, have been studied to generate drought-tolerant crops. From now on, upstream major ABA signaling components would be utilized to create drought-tolerant crops. To bring out the potential of genes involved in stress tolerance in plants, a new strategy based on the appropriate combination of genetic engineering and conventional breeding approaches would be required to develop new technologies for creating drought-tolerant crops.

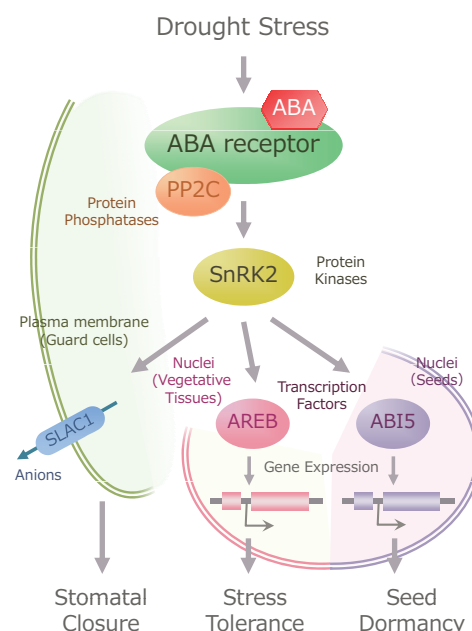


Figure: Model showing the core ABA signaling pathways in response to drought stress.

Cellular dehydration leads to an increase in endogenous ABA levels, which then activates ABA signaling pathways to cope with reduced water availability during the life of plants.

Yasunari Fujita

Biological Resources and Post-harvest Division

JIRCAS TODAY

○ Call for Nominations: 2014 Japan International Award for Young Agricultural Researchers

JIRCAS is now accepting nominations for the 2014 Japan International Award for Young Agricultural Researchers. The deadline for submitting nominations and supporting materials is on June 13, 2014 (Friday). Please visit our homepage (<http://www.jircas.affrc.go.jp/>) for detailed information, including nomination/application forms and application guidelines.

Application Guidelines for the 2014 Japan International Award for Young Agricultural Researchers

1. Purpose of the Award

This annual award is organized by the Agriculture, Forestry and Fisheries Research Council (AFFRC), the Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan, and supported by Japan International Research Center for Agricultural Sciences (JIRCAS). Its purpose is to increase motivation among young researchers contributing to research and development in agriculture, forestry, fisheries and related industries in developing countries, which is promoted by Japan for the benefit of those countries. Young researchers who show outstanding performance and research achievements that may lead to future innovation will be commended with the Award.

2. Research Fields

The award covers all research areas in agriculture, forestry, fisheries and related industries in developing countries, including the industry sectors of food, forestry and fisheries, and the fields of environmental, forestry and marine sciences. Basic research and socioeconomic fields are also covered.

3. Candidates

Candidates should be non-Japanese researchers under 40 years old as of January 01, 2014, belong to a non-Japanese research institute or a non-Japanese university, be engaged in research and development in agriculture, forestry, fisheries and related industries in developing countries, and fall under any of the following criteria (note: those who have applied for and missed the award in previous years are eligible to re-apply):

- (a) Those who have shown outstanding performance in research and development in agriculture, forestry, fisheries, or related industries for a developing country, and who show great promise.
- (b) Those who have shown outstanding achievements in research and development that will lead to future technological innovation in agriculture, forestry, fisheries or related industries for a developing country, and who show great promise.

The winning candidates must be able to attend the commendation ceremony and deliver a lecture on November 2014.

4. Number of Awardees

Up to three award winners will be named.

5. Award

The award winners will be invited to Japan to receive their awards personally and present their research results and achievements. The winners will be bestowed a testimonial by the Chairman of the AFFRC during the commendation ceremony and will also receive a cash prize of US\$5,000 each from JIRCAS.

6. How to apply

- Applications must be prepared according to the “Instructions in the Preparation of Application Forms” and submitted by the recommending institute by post. Moreover, word-processed files of each form must be submitted by the recommending institute via e-mail as they will be used for the preparation of a short-list.
- Only one researcher can be recommended from each institute, including its branches.
- If an institute recommends two candidates or more, the Secretariat will ask the institute to choose one. If the institute does not respond by the due date, all applications from that institute will be excluded.
- The application must include supporting materials that substantiate the candidate’s achievements, such as the applicant’s most important research papers (three items), technical handbooks, patent applications or utility models, newspaper articles etc., and the entire publication list directly related to the applicant’s research.
- Applications that are insufficient or incorrect will be excluded from the evaluation.

7. Determination of Winners

The award winners will be decided from among the final applicants by the Chairman of AFFRC based on the outcome of evaluation and selection carried out by the Selection Committee.

8. Criteria for Selection

The Selection Committee will give consideration to the following perspectives in evaluating and judging the applicants’ performance and research achievements for this award:

- The research and development have shown abundant originality.
- Achievements in the research and development have been objectively evaluated, e.g., by being published in

academic or technical journals.

- The research and development have provided consideration for the development of agriculture, forestry, fisheries or related industries in developing countries in terms of dissemination and commercialization.
- The research and development have shown potential for great development and for leading to future technological and methodological innovations, even when it might not be fully completed or not diffused enough at the present time.

9. Evaluation Items

The Selection Committee will evaluate the following four aspects of the applicants' work and will add general remarks which include their future potential.

(a) Evaluation Aspects and Rating (total of 20 points)

Originality (5 points): The research and development have shown originality and uniqueness. In addition to research papers, other achievements such as patent applications will also be given due consideration.

Level of research and development (5 points): Achievements in the research and development have been objectively evaluated, e.g., by being published in academic or technical journals.

Dissemination and practicability (5 points): The research has been conducted from the start with the intention of future dissemination and commercialization, in liaison with interested parties (e.g. conducting experimental studies, being given evaluation by actual beneficiaries).

Future potential (5 points): The research and development have shown potential for significant development and for leading towards future technological and methodological innovations, and are currently at a basic stage of research for future commercialization.

(b) Other Considerations

- In the case of collaborative research results, the candidate's contribution to the research will be evaluated in relation to that of the other collaborators.
- Research results created through business activities will also be properly evaluated as long as they meet the selection criteria.
- Committee members related to an institute that recommends an applicant will not participate in the assessment and rating of the applicant.
- Consideration may be given to candidates' relationship with Japan, such as research experiences in Japanese institutes and experiences of joint research with Japanese institutes.

10. Schedule

Due date for submission of application: June 13, 2014
 Selection of candidates : September 2014
 Determination of award winners : October 2014
 Date of Awarding Ceremony : November 2014

For information:

Japan International Research Center for Agricultural Sciences (JIRCAS)
<http://www.jircas.affrc.go.jp/index.html>
 E-mail address: jaward2014@ml.affrc.go.jp

Submit applications by post to:

Secretariat of the Japan International Award for Young Agricultural Researchers
 Public Relations Office (PRO)
 Japan International Research Center for Agricultural Sciences (JIRCAS)
 1-1 Ohwashi, Tsukuba City, Ibaraki
 305-8686 Japan



The 2013 awardees and selection committee members pose for a group photo following the commendation ceremony at the U Thant International Conference Hall, United Nations University in Tokyo (November 20, 2013).

JIRCAS Newsletter

Japan International Research Center for Agricultural Sciences (JIRCAS)



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