Appendix

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

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The file "Development of technologies and crops for stable food production under adverse environments and changing climate conditions" presented at the International Soybean Conference in Brazil on June 14, 2018, is attached at the end of this article. This presentation file contains information on other research related to the development of drought-tolerant GM crops. The file can be found at the following site: https://www.cbsoja.com.br/images/cbsoja2018/docs/palestras/Kazuo_Nakashima.pdf.


VIII CONGRESSO BRASILEIRO DE SOJA
June 14, 2018
Development of technologies and crops for stable food production under adverse environments and changing climate conditions
Kazuo Nakashima
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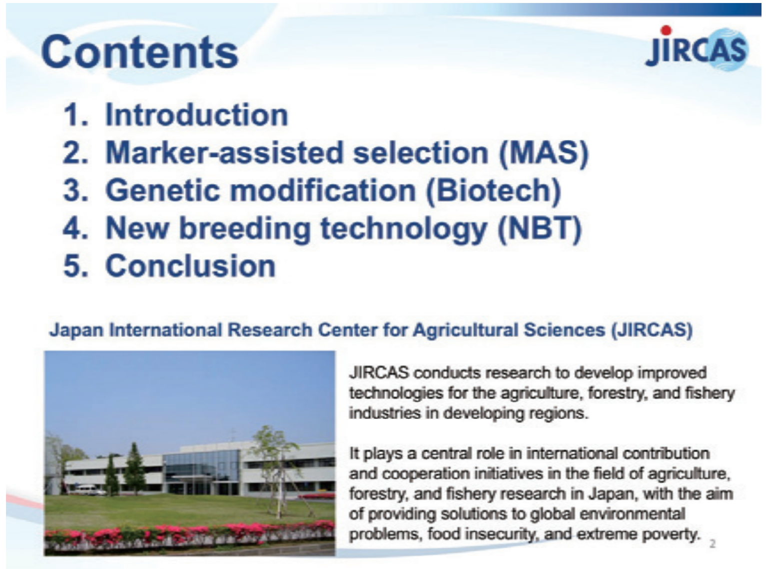
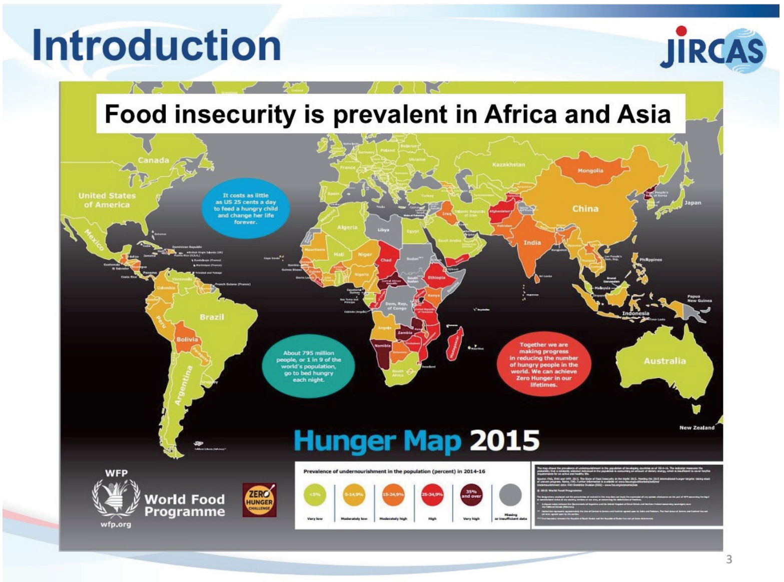
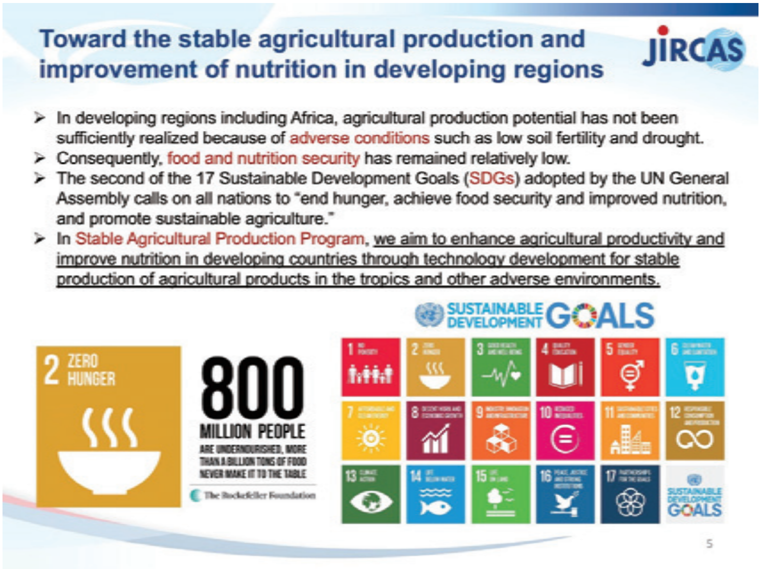
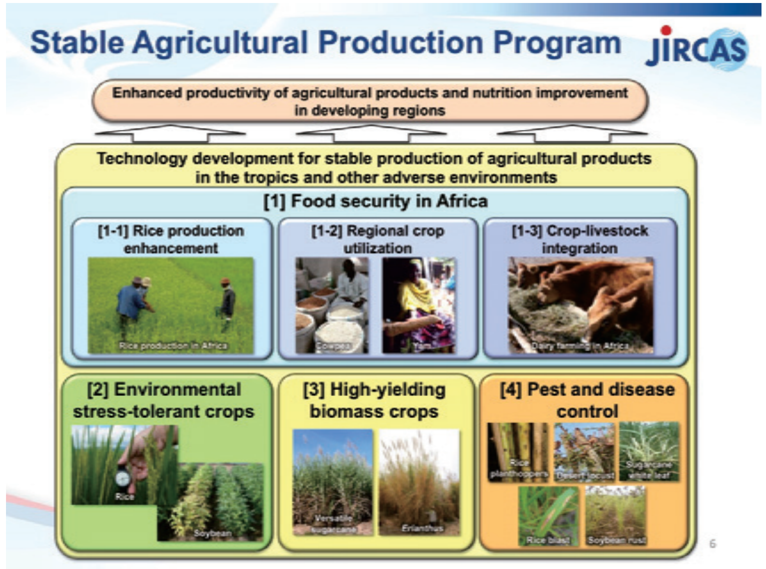
Japan International Research Center for Agricultural Sciences
JIRCAS
1

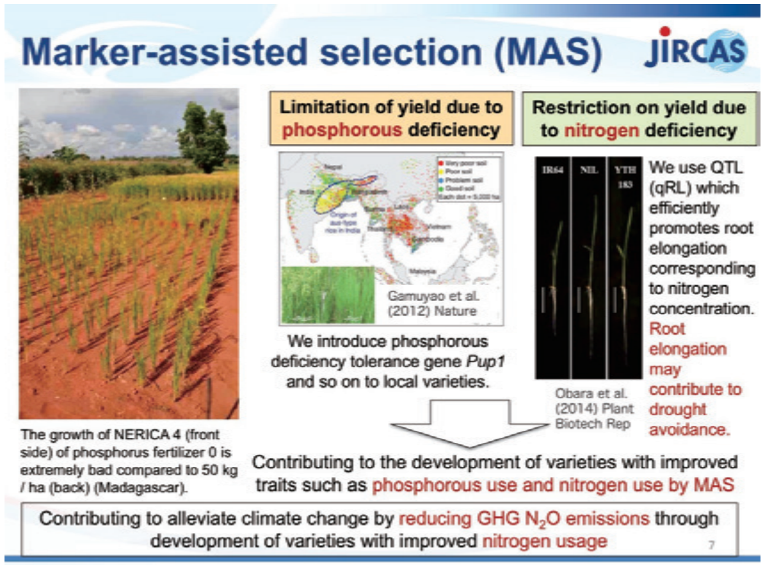

Contents
JIRCAS
1. Introduction
2. Marker-assisted selection (MAS)
3. Genetic modification (Biotech)
4. New breeding technology (NBT)
5. Conclusion
Japan International Research Center for Agricultural Sciences (JIRCAS)
JIRCAS conducts research to develop improved technologies for the agriculture, forestry, and fishery industries in developing regions.
It plays a central role in international contribution and cooperation initiatives in the field of agriculture, forestry, and fishery research in Japan, with the aim of providing solutions to global environmental problems, food insecurity, and extreme poverty.
2

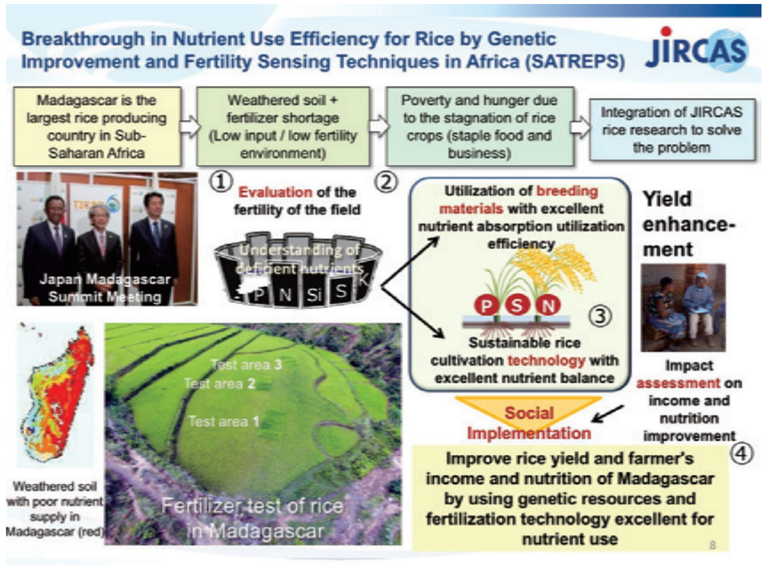

Introduction
JIRCAS
Food insecurity is prevalent in Africa and Asia
Canada
United States of America
Brazil
China
India
Australia
Hunger Map 2015
WFP
World Food Programme
wfp.org
3


Drought occurs around the world
JIRCAS
Worst drought and heat in decades disrupts life in Southeast Asia's Mekong region in 2016
http://www.nbcnews.com/slideshow/worst-drought-decades-disrupts-life-southeast-asia-s-mekong-region-n562166
4


Toward the stable agricultural production and improvement of nutrition in developing regions
JIRCAS
In developing regions including Africa, agricultural production potential has not been sufficiently realized because of adverse conditions such as low soil fertility and drought.
Consequently, food and nutrition security has remained relatively low.
The second of the 17 Sustainable Development Goals (SDGs) adopted by the UN General Assembly calls on all nations to "end hunger, achieve food security and improved nutrition, and promote sustainable agriculture."
In Stable Agricultural Production Program, we aim to enhance agricultural productivity and improve nutrition in developing countries through technology development for stable production of agricultural products in the tropics and other adverse environments.
SUSTAINABLE DEVELOPMENT GOALS
2 ZERO HUNGER
800
MILLION PEOPLE
ARE UNDERNOURISHED, MORE THAN A BILLION TONS OF FOOD NEVER MAKE IT TO THE TABLE
The Rockefeller Foundation
5

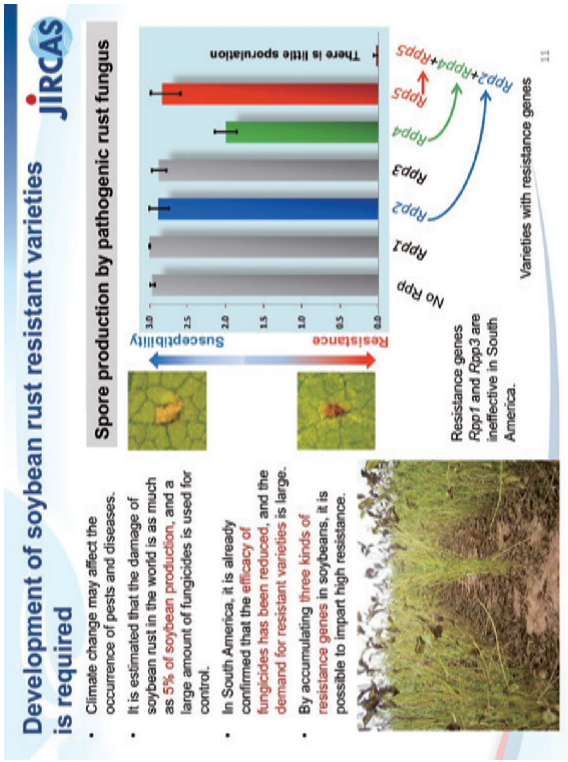

Stable Agricultural Production Program
JIRCAS
Enhanced productivity of agricultural products and nutrition improvement in developing regions
Technology development for stable production of agricultural products in the tropics and other adverse environments
[1] Food security in Africa
[1-1] Rice production enhancement
Rice production in Africa
[1-2] Regional crop utilization
Cowpea
Yam
[1-3] Crop-livestock integration
Dairy farming in Africa
[2] Environmental stress-tolerant crops
Rice
Soybean
[3] High-yielding biomass crops
Versatile sugarcane
Erianthus
[4] Pest and disease control
Rice planthoppers
Desert locust
Sugarcane white leaf
Rice blast
Soybean rust
6

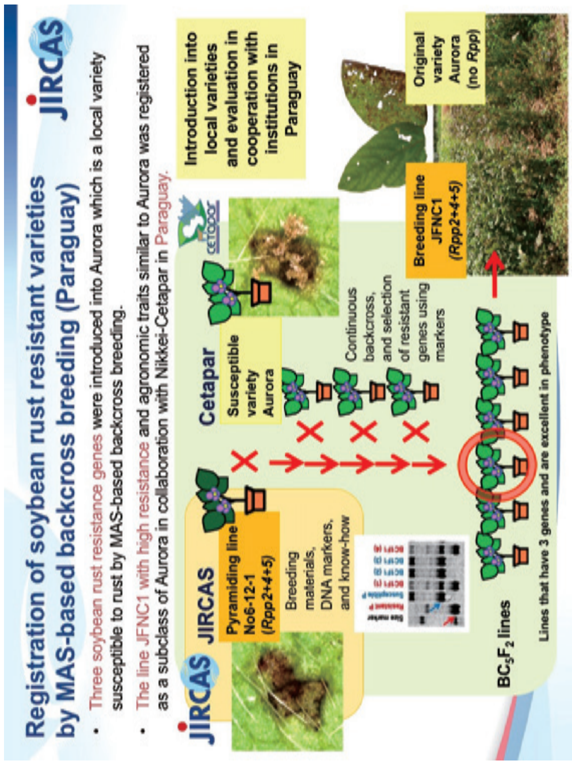

Marker-assisted selection (MAS)
JIRCAS
Limitation of yield due to phosphorous deficiency
Restriction on yield due to nitrogen deficiency
Gamuyao et al. (2012) Nature
We introduce phosphorous deficiency tolerance gene Pup1 and so on to local varieties.
IR64
NIL
YTH 183
We use QTL (qRL) which efficiently promotes root elongation corresponding to nitrogen concentration. Root elongation may contribute to drought avoidance.
Obara et al. (2014) Plant Biotech Rep
The growth of NERICA 4 (front side) of phosphorus fertilizer 0 is extremely bad compared to 50 kg / ha (back) (Madagascar).
Contributing to the development of varieties with improved traits such as phosphorous use and nitrogen use by MAS
Contributing to alleviate climate change by reducing GHG N2O emissions through development of varieties with improved nitrogen usage
7

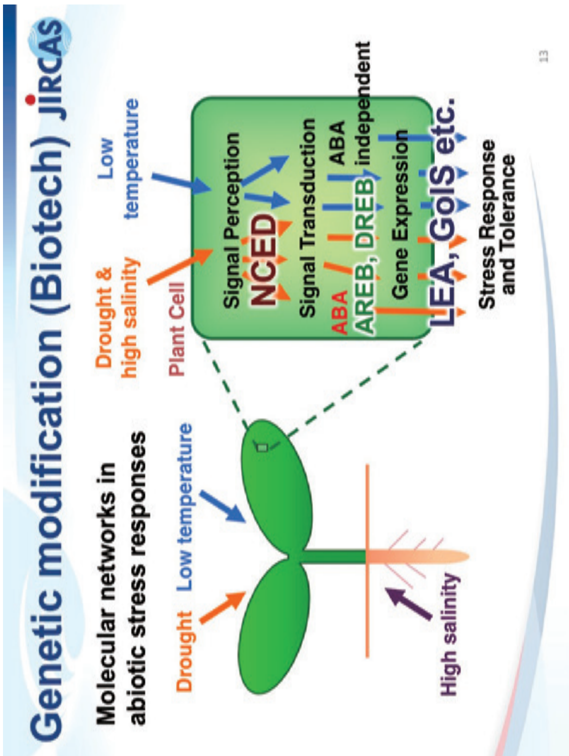

Breakthrough in Nutrient Use Efficiency for Rice by Genetic Improvement and Fertility Sensing Techniques in Africa (SATREPS)
JIRCAS
Madagascar is the largest rice producing country in Sub-Saharan Africa
Weathered soil + fertilizer shortage (Low input / low fertility environment)
Poverty and hunger due to the stagnation of rice crops (staple food and business)
Integration of JIRCAS rice research to solve the problem
Japan Madagascar Summit Meeting
① Evaluation of the fertility of the field
②
Understanding of deficient nutrients
P N Si S K
Utilization of breeding materials with excellent nutrient absorption utilization efficiency
P S N
③
Sustainable rice cultivation technology with excellent nutrient balance
Yield enhance-ment
Impact assessment on income and nutrition improvement
Social Implementation
④
Improve rice yield and farmer's income and nutrition of Madagascar by using genetic resources and fertilization technology excellent for nutrient use
Weathered soil with poor nutrient supply in Madagascar (red)
Test area 3
Test area 2
Test area 1
Fertilizer test of rice in Madagascar
8


Rice near-isogenic line (NIL) with early-morning flowering trait for improvement of heat tolerance
JIRCAS
Early-morning flowering is effective in heat escape at flowering
9


Development of salinity tolerant soybean
JIRCAS
Field performances of Ncl near isogenic lines (NILs) in a saline field condition in Japan.
Ncl could increase soybean grain yield in saline field conditions.
Do et al. (2016), Scientific Reports.
10


Development of soybean rust resistant varieties is required
JIRCAS
• Climate change may affect the occurrence of pests and diseases.
• It is estimated that the damage of soybean rust in the world is as much as 5% of soybean production, and a large amount of fungicides is used for control.
• In South America, it is already confirmed that the efficacy of fungicides has been reduced, and the demand for resistant varieties is large.
• By accumulating three kinds of resistance genes in soybeans, it is possible to impart high resistance.
Spore production by pathogenic rust fungus
Susceptibility
Resistance
3.0
2.5
2.0
1.5
1.0
0.5
0.0
There is little sporulation
No Rpp
Rpp1
Rpp2
Rpp3
Rpp4
Rpp5
Rpp2+Rpp4+Rpp5
Resistance genes Rpp1 and Rpp3 are ineffective in South America.
Varieties with resistance genes
11


Registration of soybean rust resistant varieties by MAS-based backcross breeding (Paraguay)
JIRCAS
• Three soybean rust resistance genes were introduced into Aurora which is a local variety susceptible to rust by MAS-based backcross breeding.
• The line JFNC1 with high resistance and agronomic traits similar to Aurora was registered as a subclass of Aurora in collaboration with Nikkei-Cetapar in Paraguay.
JIRCAS
JIRCAS
Pyramiding line No6-12-1 (Rpp2+4+5)
Breeding materials, DNA markers, and know-how
Cetapar
Susceptible variety Aurora
Continuous backcross, and selection of resistant genes using markers
Introduction into local varieties and evaluation in cooperation with institutions in Paraguay
BC5F2 lines
Lines that have 3 genes and are excellent in phenotype
Breeding line JFNC1 (Rpp2+4+5)
Original variety Aurora (no Rpp)


Genetic modification (Biotech)
JIRCAS
Molecular networks in abiotic stress responses
Drought
Low temperature
High salinity
Drought & high salinity
Low temperature
Plant Cell
Signal Perception
NCED
Signal Transduction
ABA
ABA
AREB, DREB
independent
Gene Expression
LEA, GolS etc.
Stress Response and Tolerance
13


Drought tolerance of the transgenic plants
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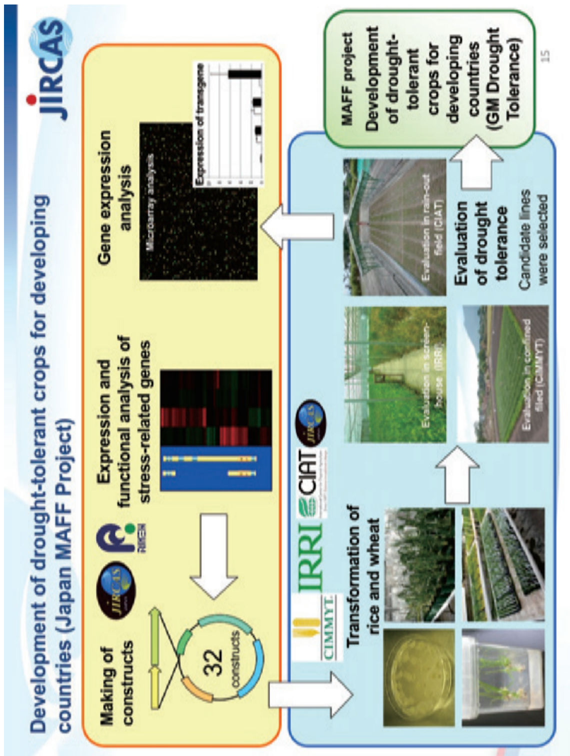
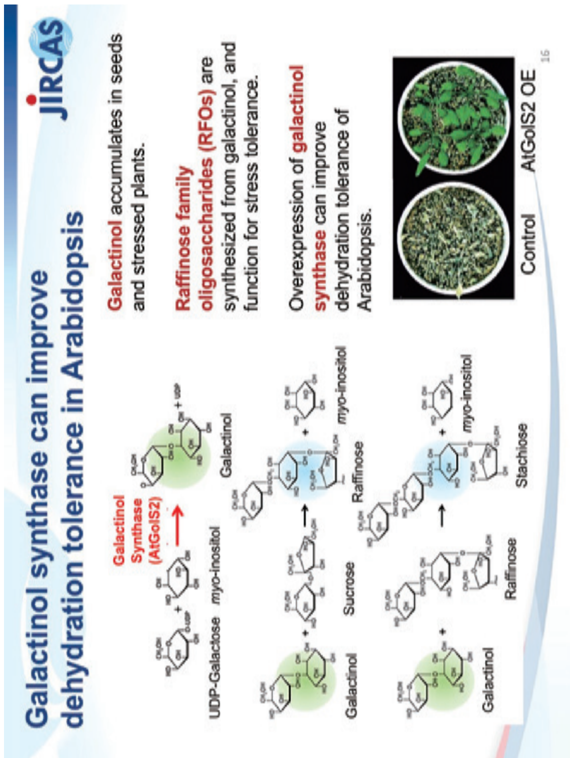
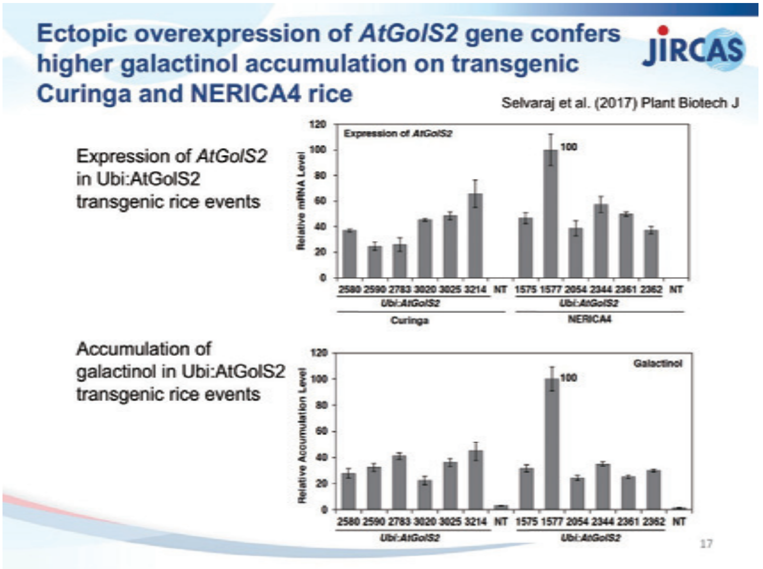
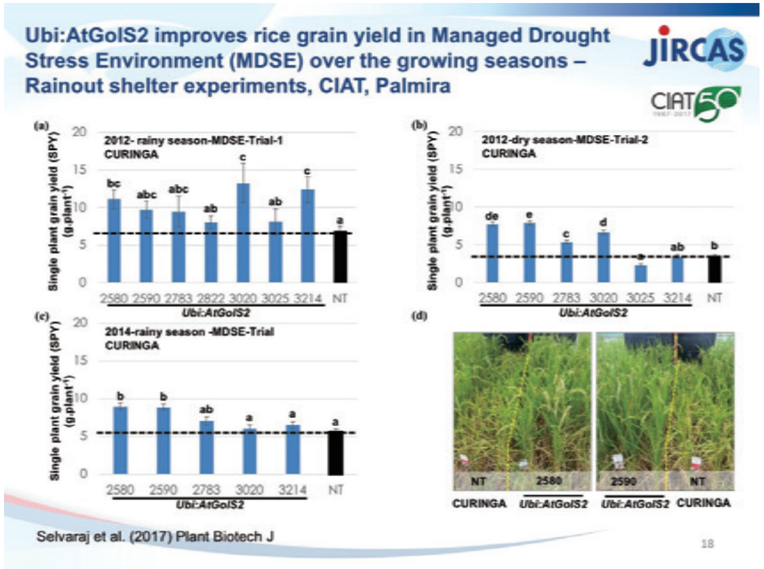
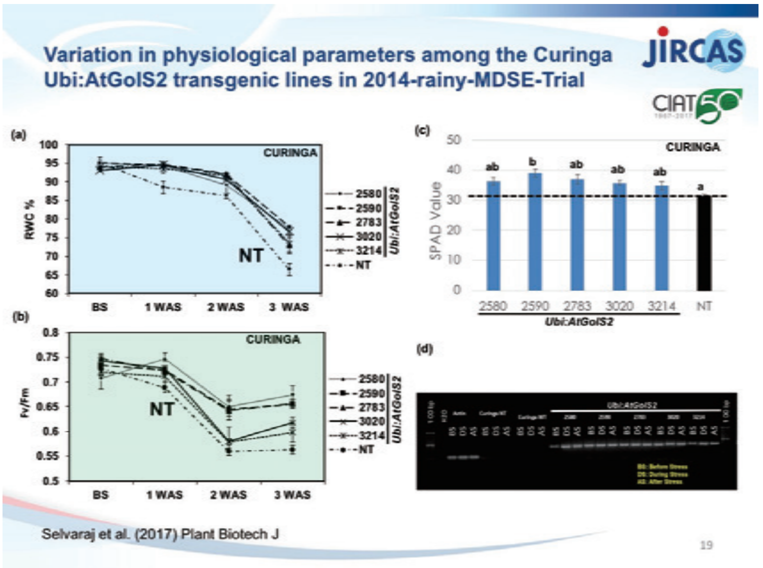
Rice (JIRCAS)
Wheat (CIMMYT)
Soybean (Embrapa)
Groundnut (ICRISAT)
14


Development of drought-tolerant crops for developing countries (Japan MAFF Project)
JIRCAS
Making of constructs
32 constructs
Expression and functional analysis of stress-related genes
Gene expression analysis
Microarray analysis
Expression of transgene
CIMMYT
IRRI
CIAT
Transformation of rice and wheat
Evaluation in screen-house (IRRI)
Evaluation in confined filed (CIMMYT)
Evaluation in rain-out field (CIAT)
Evaluation of drought tolerance
Candidate lines were selected
MAFF project
Development of drought-tolerant crops for developing countries (GM Drought Tolerance)
15


Galactinol synthase can improve dehydration tolerance in Arabidopsis
JIRCAS
UDP-Galactose
myo-inositol
Galactinol Synthase (AtGolS2)
Galactinol
Sucrose
Raffinose
myo-inositol
Stachiose
Galactinol accumulates in seeds and stressed plants.
Raffinose family oligosaccharides (RFOs) are synthesized from galactinol, and function for stress tolerance.
Overexpression of galactinol synthase can improve dehydration tolerance of Arabidopsis.
Control
AtGolS2 OE
16


Ectopic overexpression of AtGolS2 gene confers higher galactinol accumulation on transgenic Curinga and NERICA4 rice
JIRCAS
Selvaraj et al. (2017) Plant Biotech J
Expression of AtGolS2 in Ubi:AtGolS2 transgenic rice events
Expression of AtGolS2
Relative mRNA Level
2580 2590 2783 3020 3025 3214 NT 1575 1577 2054 2344 2361 2362 NT
Ubi:AtGolS2
Curinga
NERICA4
Accumulation of galactinol in Ubi:AtGolS2 transgenic rice events
Galactinol
Relative Accumulation Level
17


Ubi:AtGolS2 improves rice grain yield in Managed Drought Stress Environment (MDSE) over the growing seasons – Rainout shelter experiments, CIAT, Palmira
JIRCAS
CIAT
2012- rainy season-MDSE-Trial-1
CURINGA
2012-dry season-MDSE-Trial-2
CURINGA
2014-rainy season -MDSE-Trial
CURINGA
Single plant grain yield (SPY) (g.plant-1)
Ubi:AtGolS2
NT 2580 2590 NT
CURINGA Ubi:AtGolS2 Ubi:AtGolS2 CURINGA
Selvaraj et al. (2017) Plant Biotech J
18


Variation in physiological parameters among the Curinga Ubi:AtGolS2 transgenic lines in 2014-rainy-MDSE-Trial
JIRCAS
CIAT
CURINGA
RWC %
Fv/Fm
NT
BS 1 WAS 2 WAS 3 WAS
2580 2590 2783 3020 3214 NT
Ubi:AtGolS2
SPAD Value
Selvaraj et al. (2017) Plant Biotech J
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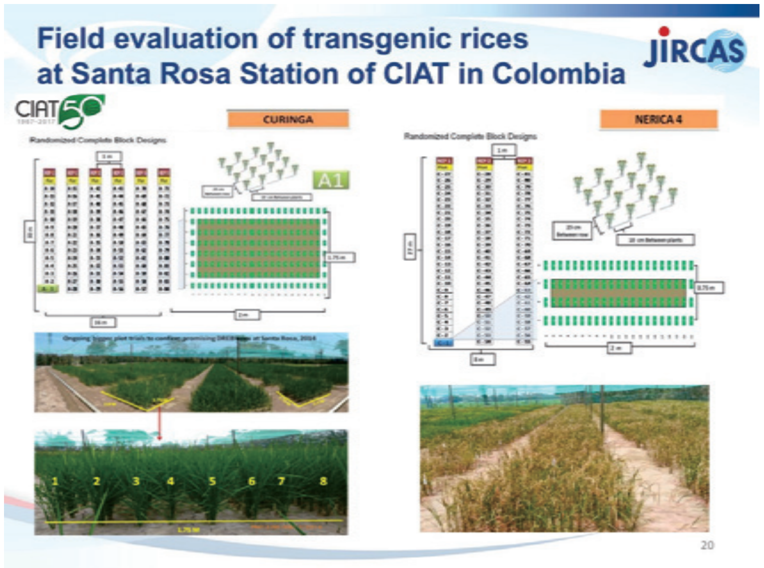
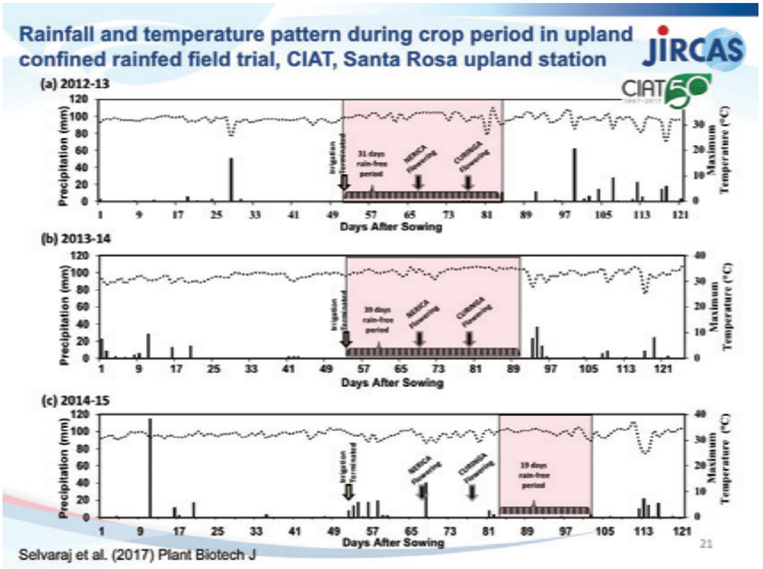
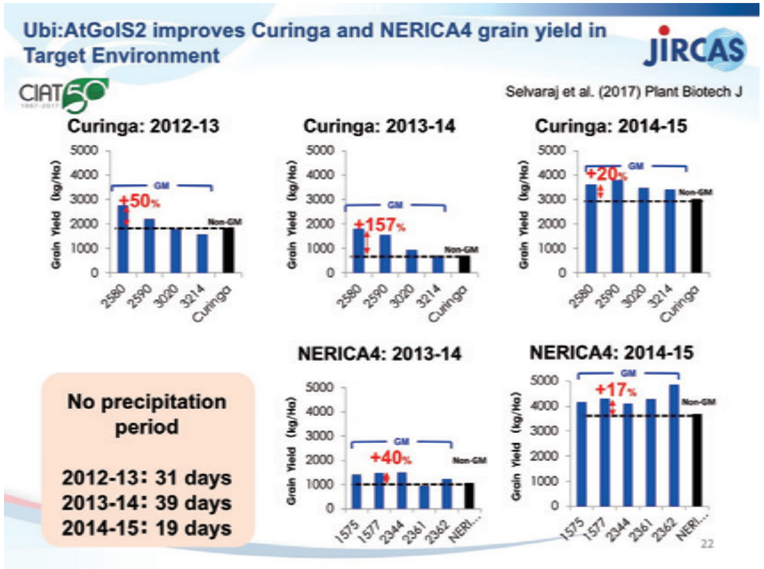
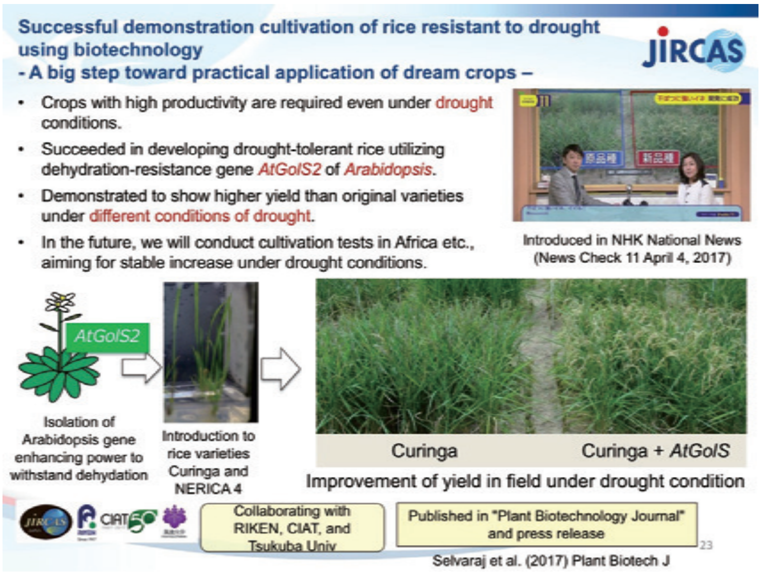
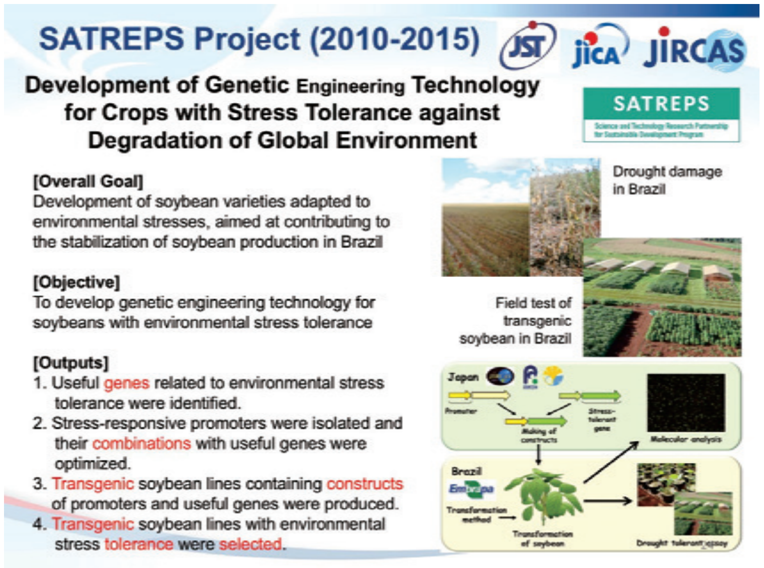
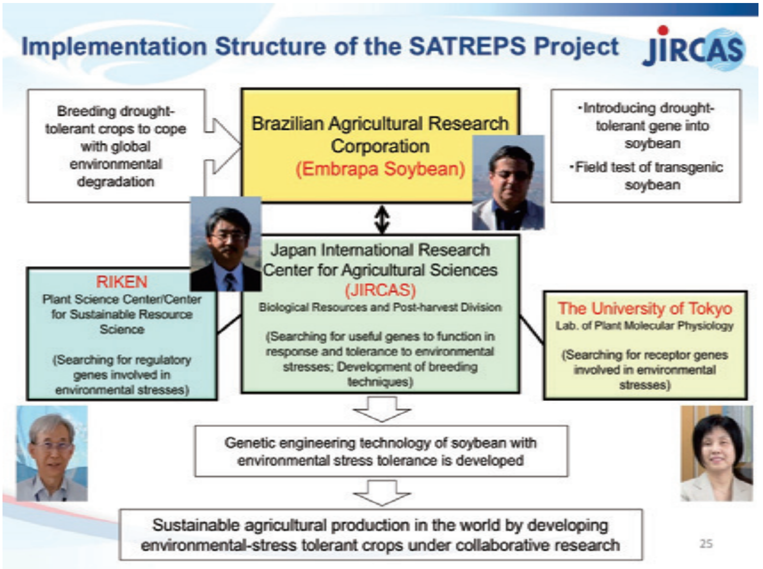

Field evaluation of transgenic rices at Santa Rosa Station of CIAT in Colombia
JIRCAS
CIAT
CURINGA
NERICA 4
Randomized Complete Block Designs
A1
20


Rainfall and temperature pattern during crop period in upland confined rainfed field trial, CIAT, Santa Rosa upland station
JIRCAS
CIAT 50
(a) 2012-13
(b) 2013-14
(c) 2014-15
Precipitation (mm)
Maximum Temperature (°C)
Days After Sowing
Irrigation Terminated
31 days rain-free period
39 days rain-free period
19 days rain-free period
NERICA flowering
CURINGA flowering
Selvaraj et al. (2017) Plant Biotech J
21


Ubi:AtGolS2 improves Curinga and NERICA4 grain yield in Target Environment
JIRCAS
CIAT 50
Selvaraj et al. (2017) Plant Biotech J
Curinga: 2012-13
Curinga: 2013-14
Curinga: 2014-15
NERICA4: 2013-14
NERICA4: 2014-15
Grain Yield (kg/Ha)
GM
Non-GM
+50%
+157%
+20%
+40%
+17%
2580
2590
3020
3214
Curinga
1575
1577
2344
2361
2362
NERI...
No precipitation period
2012-13: 31 days
2013-14: 39 days
2014-15: 19 days
22


Successful demonstration cultivation of rice resistant to drought using biotechnology
- A big step toward practical application of dream crops –
JIRCAS
• Crops with high productivity are required even under drought conditions.
• Succeeded in developing drought-tolerant rice utilizing dehydration-resistance gene AtGolS2 of Arabidopsis.
• Demonstrated to show higher yield than original varieties under different conditions of drought.
• In the future, we will conduct cultivation tests in Africa etc., aiming for stable increase under drought conditions.
Introduced in NHK National News (News Check 11 April 4, 2017)
AtGolS2
Isolation of Arabidopsis gene enhancing power to withstand dehydation
Introduction to rice varieties Curinga and NERICA 4
Curinga
Curinga + AtGolS
Improvement of yield in field under drought condition
Collaborating with RIKEN, CIAT, and Tsukuba Univ
Published in "Plant Biotechnology Journal" and press release
Selvaraj et al. (2017) Plant Biotech J
23


SATREPS Project (2010-2015)
JST
JICA
JIRCAS
Development of Genetic Engineering Technology for Crops with Stress Tolerance against Degradation of Global Environment
SATREPS
[Overall Goal]
Development of soybean varieties adapted to environmental stresses, aimed at contributing to the stabilization of soybean production in Brazil
[Objective]
To develop genetic engineering technology for soybeans with environmental stress tolerance
[Outputs]
1. Useful genes related to environmental stress tolerance were identified.
2. Stress-responsive promoters were isolated and their combinations with useful genes were optimized.
3. Transgenic soybean lines containing constructs of promoters and useful genes were produced.
4. Transgenic soybean lines with environmental stress tolerance were selected.
Drought damage in Brazil
Field test of transgenic soybean in Brazil
Japan
Promoter
Making of constructs
Stress-tolerant gene
Molecular analysis
Brazil
Transformation method
Transformation of soybean
Drought tolerant assay


Implementation Structure of the SATREPS Project
JIRCAS
Breeding drought-tolerant crops to cope with global environmental degradation
Brazilian Agricultural Research Corporation (Embrapa Soybean)
・Introducing drought-tolerant gene into soybean
・Field test of transgenic soybean
RIKEN
Plant Science Center/Center for Sustainable Resource Science
(Searching for regulatory genes involved in environmental stresses)
Japan International Research Center for Agricultural Sciences (JIRCAS)
Biological Resources and Post-harvest Division
(Searching for useful genes to function in response and tolerance to environmental stresses; Development of breeding techniques)
The University of Tokyo
Lab. of Plant Molecular Physiology
(Searching for receptor genes involved in environmental stresses)
Genetic engineering technology of soybean with environmental stress tolerance is developed
Sustainable agricultural production in the world by developing environmental-stress tolerant crops under collaborative research
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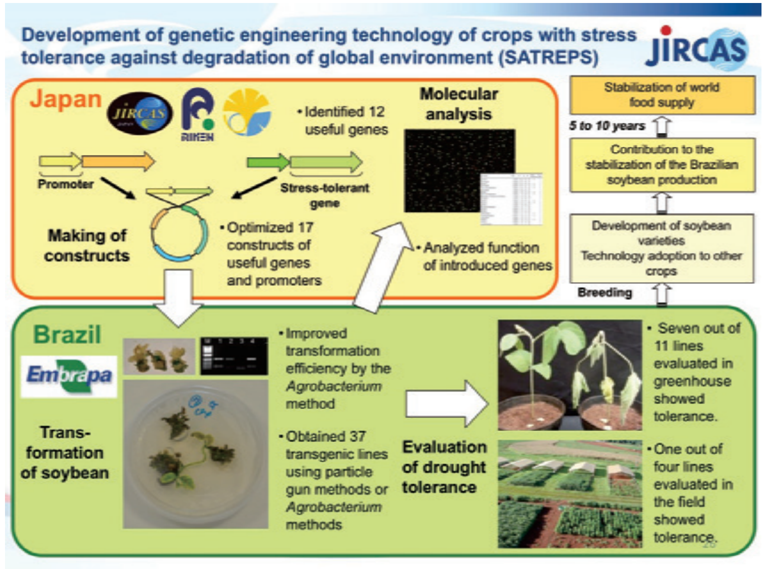
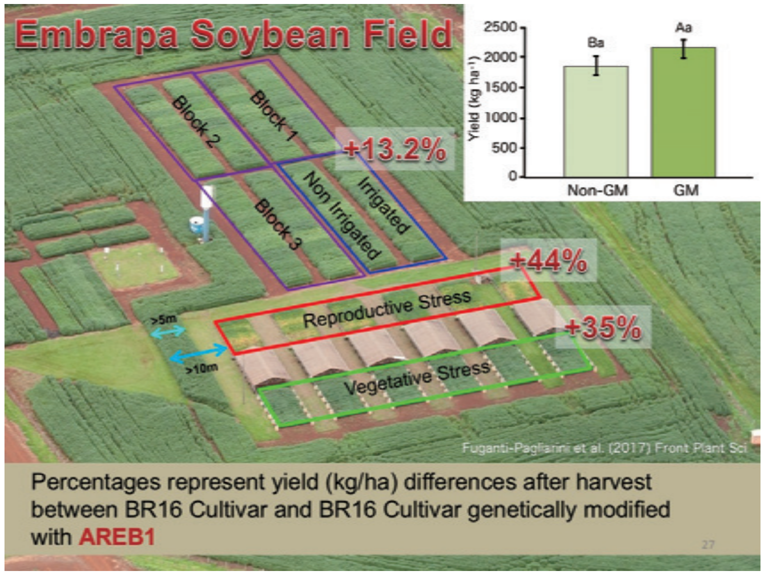
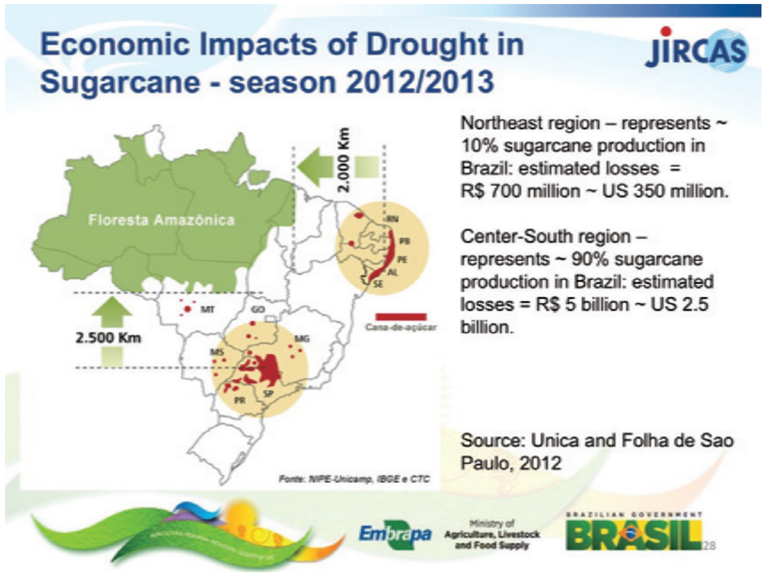
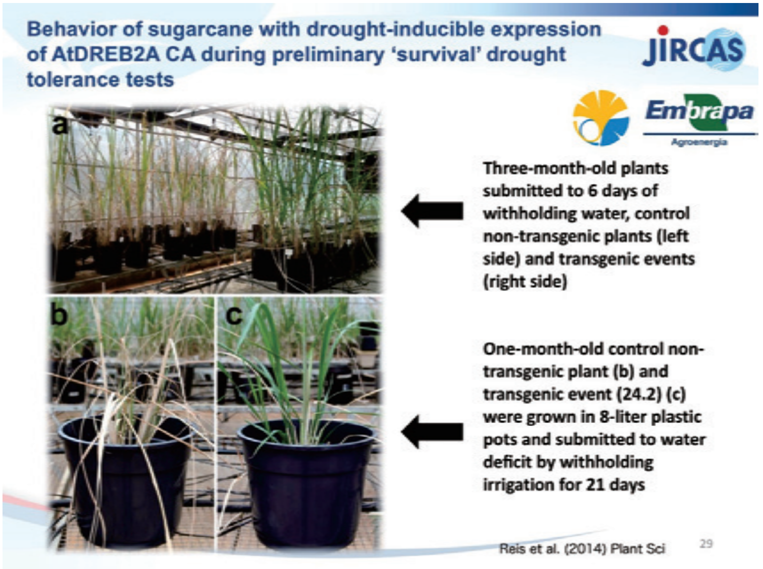
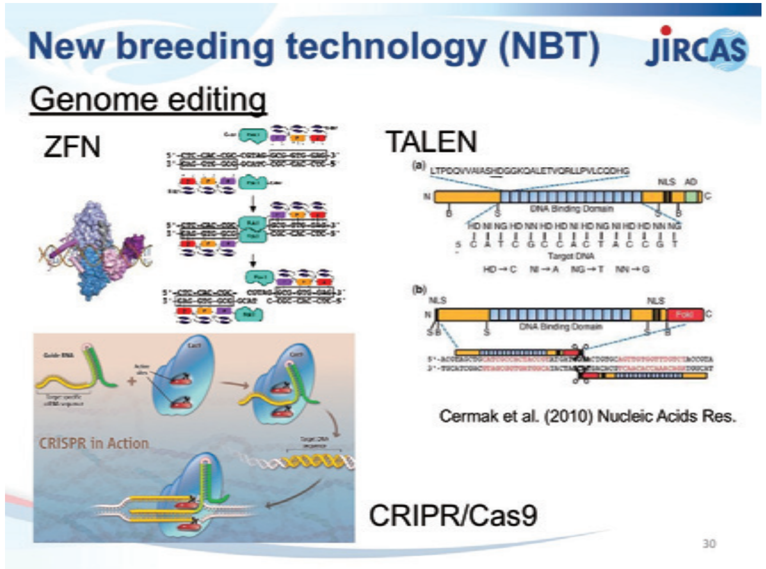
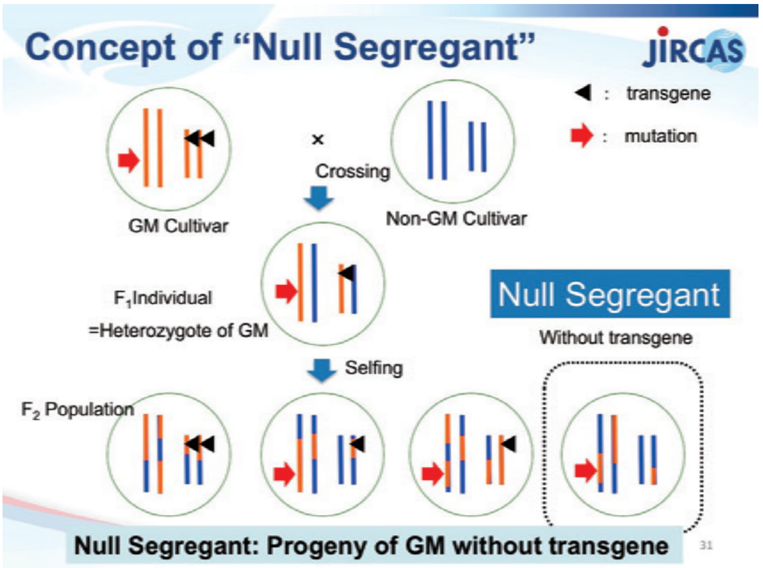

Development of genetic engineering technology of crops with stress tolerance against degradation of global environment (SATREPS)
JIRCAS
Japan
Promoter
Stress-tolerant gene
Making of constructs
・Identified 12 useful genes
・Optimized 17 constructs of useful genes and promoters
Molecular analysis
・Analyzed function of introduced genes
Stabilization of world food supply
5 to 10 years
Contribution to the stabilization of the Brazilian soybean production
Development of soybean varieties
Technology adoption to other crops
Breeding
Brazil
Embrapa
Trans-formation of soybean
・Improved transformation efficiency by the Agrobacterium method
・Obtained 37 transgenic lines using particle gun methods or Agrobacterium methods
Evaluation of drought tolerance
・Seven out of 11 lines evaluated in greenhouse showed tolerance.
・One out of four lines evaluated in the field showed tolerance.

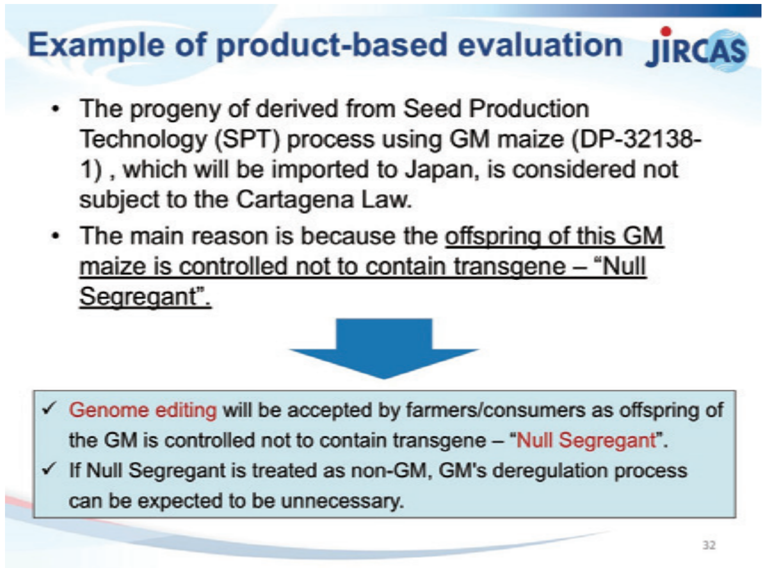

Embrapa Soybean Field
Block 1
Block 2
Block 3
Irrigated
Non Irrigated
+13.2%
+44%
+35%
Reproductive Stress
Vegetative Stress
>5m
>10m
Yield (kg ha⁻¹)
2500
2000
1500
1000
500
0
Ba
Aa
Non-GM
GM
Fuganti-Pagliarini et al. (2017) Front Plant Sci
Percentages represent yield (kg/ha) differences after harvest between BR16 Cultivar and BR16 Cultivar genetically modified with AREB1
27


Economic Impacts of Drought in Sugarcane - season 2012/2013
JIRCAS
Floresta Amazônica
2.000 Km
2.500 Km
Cana-de-açúcar
Fonte: NIPE-Unicamp, IBGE e CTC
Northeast region – represents ~ 10% sugarcane production in Brazil: estimated losses = R$ 700 million ~ US 350 million.
Center-South region – represents ~ 90% sugarcane production in Brazil: estimated losses = R$ 5 billion ~ US 2.5 billion.
Source: Unica and Folha de Sao Paulo, 2012
Embrapa
Ministry of Agriculture, Livestock and Food Supply
BRAZILIAN GOVERNMENT
BRASIL
28


Behavior of sugarcane with drought-inducible expression of AtDREB2A CA during preliminary 'survival' drought tolerance tests
JIRCAS
Embrapa
Agroenergia
a
b
c
Three-month-old plants submitted to 6 days of withholding water, control non-transgenic plants (left side) and transgenic events (right side)
One-month-old control non-transgenic plant (b) and transgenic event (24.2) (c) were grown in 8-liter plastic pots and submitted to water deficit by withholding irrigation for 21 days
Reis et al. (2014) Plant Sci
29


New breeding technology (NBT)
JIRCAS
Genome editing
ZFN
TALEN
CRISPR in Action
CRIPR/Cas9
Cermak et al. (2010) Nucleic Acids Res.
30


Concept of "Null Segregant"
JIRCAS
: transgene
: mutation
GM Cultivar
×
Crossing
Non-GM Cultivar
F1Individual
=Heterozygote of GM
Selfing
F2 Population
Null Segregant
Without transgene
Null Segregant: Progeny of GM without transgene
31


Example of product-based evaluation
JIRCAS
• The progeny of derived from Seed Production Technology (SPT) process using GM maize (DP-32138-1) , which will be imported to Japan, is considered not subject to the Cartagena Law.
• The main reason is because the offspring of this GM maize is controlled not to contain transgene – "Null Segregant".
✓ Genome editing will be accepted by farmers/consumers as offspring of the GM is controlled not to contain transgene – "Null Segregant".
✓ If Null Segregant is treated as non-GM, GM's deregulation process can be expected to be unnecessary.
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Genome editing systems in rice cultivars and strategy for producing desired mutants with homozygous mutation

JIRCAS

CRISPR/Cas9 is a novel tool for targeted mutagenesis. *Agrobacterium*-mediated methods using immature embryos successfully transformed a CRISPR/Cas9 system into five rice cultivars and subsequently induced mutation.

Table 1. Targeted mutagenesis in rice cultivars using the CRISPR/Cas9 system and the *Agrobacterium*-mediated transformation of immature embryos

Cultivar	Number of transgenic plants	Number of monoallelic mutants	Number of biallelic mutants	Total number of mutants
Nipponbare	106	26	64	90
Koshihikari	17	1	10	11
NERICA1	54	16	33	49
Curinga	272	137	80	217
IR64	5	4	1	5
Total	454	184	188	372

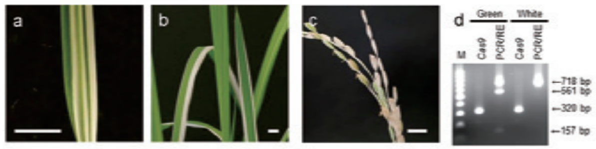

Fig. 1. Diagrammatic model showing the unpredicted segregation of a targeted mutation

Ishizaki T (2016) Mol. Breeding

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Virus-induced down-regulation will accelerate the functional analysis of stress-related gene

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ALSV-induced down-regulation of *GmERA1* genes enhances the stomatal response to abscisic acid and drought resistance in soybean.

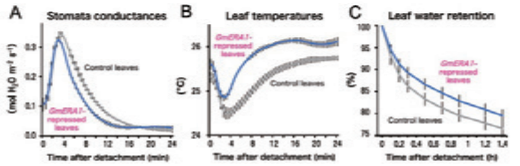
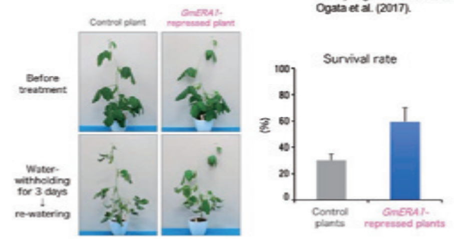


Fig. 1. Improved drought stress responses in GmERA1-repressed soybean leaves
Leaf detachment-induced response of stomatal conductance (A), leaf temperature (B), and water retention (C) in soybean leaves infected with GmERA1-recombinant virus were measured. Data are means ± SE (n = 3 to 6). Figures are modified from Ogata et al. (2017).

Fig. 2. Improved drought tolerance in GmERA1-repressed soybean plants
The soybean plants infected with GmERA1-recombinant virus were withheld water for three days before re-watering (left panels). Survival rate of the plants were recorded after re-watering (right panel). Data are means ± SE (n = 3 to 6). Figures are modified from Ogata et al. (2017).



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Conclusion

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1. To ensure food and nutrition security, we will endeavor to develop technologies and crops with high productivity and adaptability to adverse environments and changing climate conditions.
2. MAS : We have developed near-isogenic lines (NILs) with stress tolerance of crops such as soybean and rice using MAS.
3. Biotech: We have shown that overexpression of genes encoding stress-related transcription factors (e.g., DREB, AREB) and enzymes (e.g., galactinol synthase) improved drought tolerance in transgenic crops such as soybean and rice.
4. NBT: We are challenging to generate stress-tolerant crops utilizing NBT such as genome editing.
5. We hope these technologies and materials could contribute to achieving food and nutrition security in developing regions.

Important activity for researchers:
Public relations and two-way communication
Example: Civic participation type of two-way communication

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Acknowledgement

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MAS

N Rice: Obara M, Fukuta Y (JIRCAS), Ishimaru T (JIRCAS/NARO), Kobayashi N, Fujita D (NARO) et al.
P Rice: Wissuwa M, Juan P-T (JIRCAS), Gamuyao R, Hyoun CJ, Catausan S, Dalid C, Slamet-Loedin I, Heuer S. (IRRI)、Pesaresi P (Univ of Milano)、Tecson-Mendoza EM (Univ of Philippines, Los Baño) et al.
SATREPS Madagascar: Tsujimoo Y, Wissuwa M, Yokoyama S (JIRCAS), Moritsuka N (Univ of Kyoto)、Razafimbelo T (LRI, Univ of Antananarivo)、Ramanantsoanirina A, Raymond R, Abel-Ratovo H (FOFIFA) et al.
EMF Rice: Ishimaru T (JIRCAS/NARO), Hirabayashi H, Kobayashi N, Fujita D (NARO) , Sasaki K (Univ of Tokyo), Gannaban RB, Simon EV, Lumanglas PD, Jagadish KSV (IRRI), Miras MA, Mendioro MS (U o Philippines, Los Baño) et al.
NaCl Soybean: Xu D, Shono M, Suenaga K, Do TD, Chen H, Vu HTT, Hamwieh A (JIRCAS), Yamada T (Hokkaido Univ), Sato T (Tohoku Univ), Yan Y, Cong H (Xinjiang Academy of Agricultural Sciences) et al.
Rust Soybean: Yamanaka N (JIRCAS), Shimakawa MU, Espinola C, Ohyama I (Nikkei-Cetapar, Paraguay)

Biotech

Rice: Selvaraj MG, Ishitani M (CIAT), Shinozaki K (RIKEN), Kusano M (Univ of Tsukuba), Ishizaki T (JIRCAS) et al.
Soybean: Nepomuceno AL (Embrapa Soybean), Yamaguchi-Shinozaki K (Univ of Tokyo) , Kanamori N (JIRCAS) et al.
Sugarcane: Molinari HBC (Embrapa Agroenergy) , Yamaguchi-Shinozaki K (Univ of Tokyo) et al.

NBT

CRISPR/Cas9 Rice: Ishizaki T (JIRCAS)
VIGS Soybean : Ogata T, Nagatoshi Y, , Fujita Y (JIRCAS) Yamagishi N, Yoshikawa N (Iwate Univ)

MAFF JST JICA RIKEN Embrapa NARO IRRI CIAT

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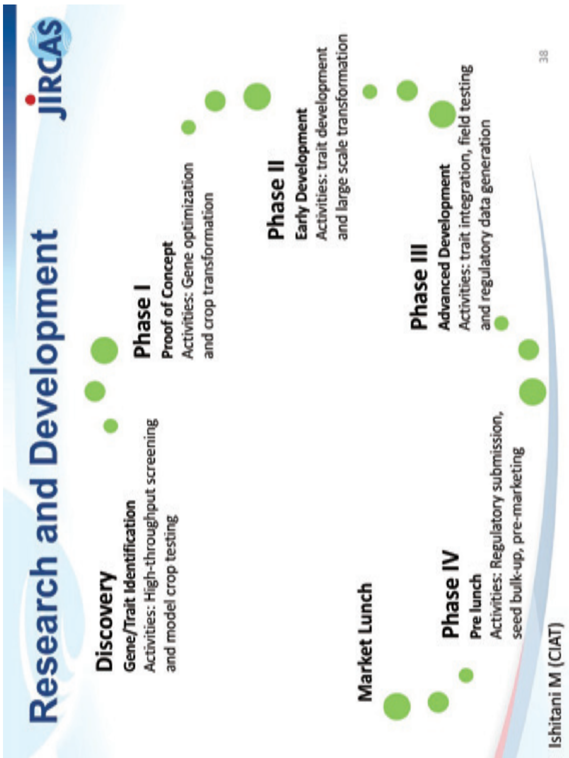
Research and Development
JIRCAS
Discovery
Gene/Trait Identification
Activities: High-throughput screening and model crop testing
Phase I
Proof of Concept
Activities: Gene optimization and crop transformation
Phase II
Early Development
Activities: trait development and large scale transformation
Phase III
Advanced Development
Activities: trait integration, field testing and regulatory data generation
Phase IV
Pre lunch
Activities: Regulatory submission, seed bulk-up, pre-marketing
Market Lunch
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