

Strategies and Practice toward the Development of Green Super Rice

Qifa Zhang

National Key Laboratory of Crop Genetic Improvement
Huazhong Agricultural University
Wuhan, China

Challenges to rice production

- Severe damages by pests and diseases, and indiscriminate applications of pesticides
- Pressure for high yield and over use of chemical fertilizers
- Water shortage and drought
- Low productivity of marginal lands
- Pressures for improvement of grain quality
- Ever increasing demands for increasing yield potential

Damages by insects and diseases and pesticide use in rice

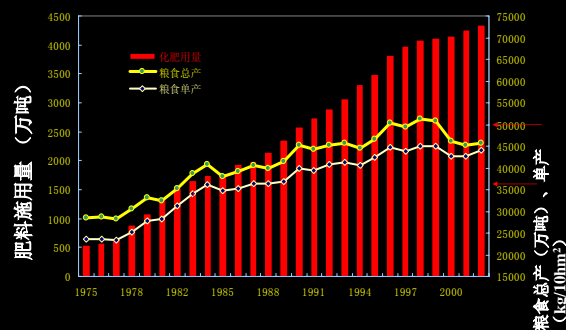
- Major pests
 - Leaf-folders and stemborers
 - Plant hoppers
- Major diseases
 - Blast
 - Sheath blight
 - Leaf blight
- Excessive application of chemical pesticides
 - Severe damages to the environments
 - Heavy economic costs to the farmers
 - Harms to the health of farmers and consumers



Consumption of nitrogen and phosphorus fertilizers in China

- Nitrogenous fertilizers (<http://faostat.fao.org/>) :
 - 25.43 million tons in 2002
 - 30.0% of world total (84.75 million tons)
- Phosphate fertilizers (<http://faostat.fao.org/>) :
 - 9.92 million tons in 2002
 - 29.6% of the world total (33.55 million tons)
- The total consumption is projected to be 60 million tons by 2030 (国家中长期肥料发展规划)

Trends of grain production and fertilizer consumption in China in the last 30 years

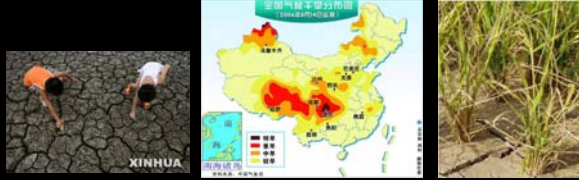


Negative impacts of over-fertilization

- Low efficiency of utilization
- Eutrophication of ground water, rivers, lakes and the seas
- High cost to the farmers
- Reducing the quality of the products

Water use and drought in rice

- Agriculture uses about 70% of the water consumed in China, of which about 70% is used for rice production
- Drought is occurring more frequently than ever before



Chinese scientists call for a “second green revolution”

- The goals of “second green revolution”
 - Less input
 - More production
 - Better environment
- 少投入、多产出、保护环境

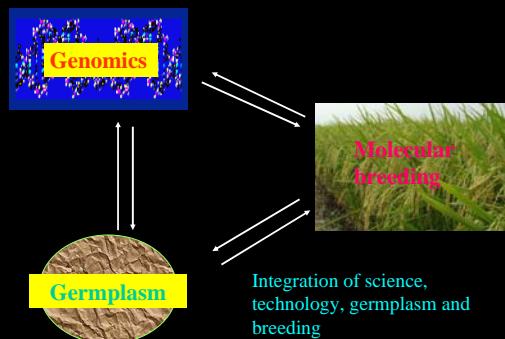
Green Super Rice

- Pesticide spray minimized
- Fertilizer application greatly reduced
- Water saving and drought resistant
- Superior quality
- High yielding

Three stages of development

- Minimizing pesticide spray (3-4 years)
- Large reduction in fertilizer application (5-8 years)
- Drought resistance (8-10 years)

The strategy of the program



GOAL 1: Minimizing pesticide use

- Adequate genetic (gene) resources
- Technology matured
- Lines and cultivars already available
- Main constraints are in policies and extension systems



In the pipeline: additional genes and transgenics

- More genes
 - Cry1Ab
 - Cry1Ac
 - Cry1Ca
 - Cry2Ab
 - Cry9C
 - Shuffling products of these genes
- Transgenic rice with multiple resistances as a strategy for resistance management



Transgenic rice with *Bt* gene driven by a green tissue specific promoter

$P_{rbcs}:GUS$: green tissue specific

$P_{rbcs}:Cry1C$: transgenic rice

Lin et al. unpublished

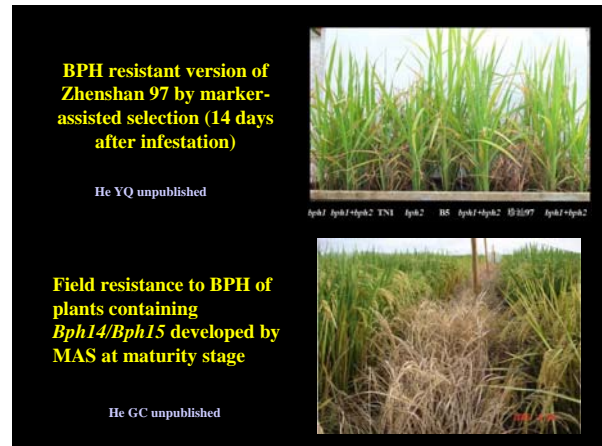
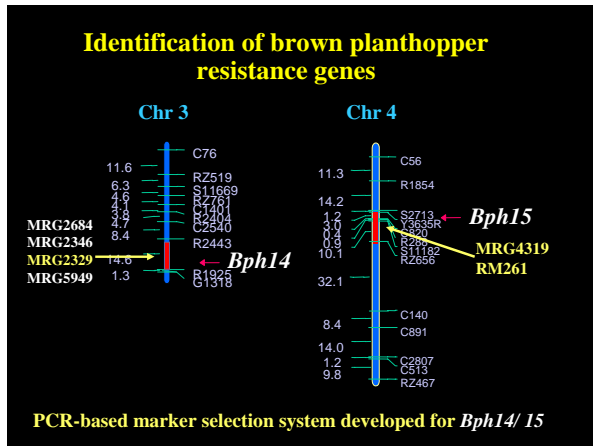
40 days after artificial infestation of yellow stem borer

Brown planthopper resistant genes reported in the literature

Zhang Q 2007 PNAS 104:16402-16409

Qianjiang, Hubei, by GC He

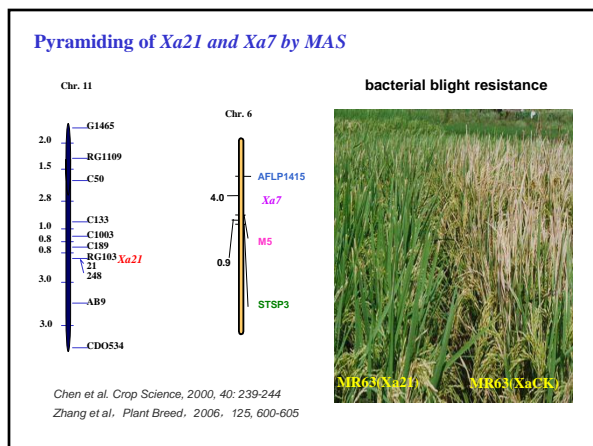
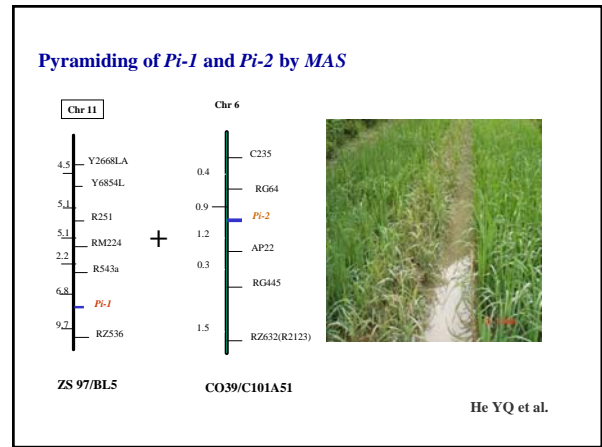
Germplasm	Gene	Chrom	Reaction to biotype				Reference
			1	2	3	4	
Mudgo	<i>Bph1</i>	12	R	S	R	S	36-38
ASD7	<i>Bph2</i>	12	R	R	S	S	36, 39
Rathu Heenati	<i>Bph3</i>	6	R	R	R	R	40
Babawee	<i>Bph4</i>	6	R	R	R	R	41, 42
ARC 10550	<i>Bph5</i>		S	S	S	R	43
Swarnalata	<i>Bph6</i>		S	S	S	R	43
T12	<i>Bph7</i>		S	S	S	R	43
Chin Saba	<i>Bph8</i>		R	R	R	-	44
Balamawee	<i>Bph9</i>	12	R	R	R	-	44, 45
<i>O. australiensis</i>	<i>Bph10</i>	12	R	R	R	-	46
<i>O. officinalis</i>	<i>Bph11</i>	9	-	-	-	-	47
<i>O. officinalis</i>	<i>Bph12</i>	3	-	-	-	-	48
<i>O. eichinger</i>	<i>Bph13</i>	2	R	R	-	-	49
B5	<i>Bph14</i>	3	R	R	-	-	50
B5	<i>Bph15</i>	4	R	R	-	-	50
<i>O. officinalis</i>	<i>Bph16</i>	4	-	-	-	-	51
B14	<i>Bph17</i>	4	R	R	-	-	52
<i>O. officinalis</i>	<i>Bph17</i>	3	-	-	-	R	53
<i>O. australiensis</i>	<i>Bph18</i>	12	BPH pop on Taebuckbyeo	-	-	-	54
AS20-1	<i>Bph19</i>	3	-	R	-	-	55



Reactions of NILs to isolates of *Pyricularia grisea*

NILs	Gene	Infection strains	Resistance frequency (%)
C101LAC	<i>Pi1</i>	13	82.7
C101A51	<i>Pi2</i>	11	85.3
C104PKT	<i>Pi3</i>	57	24.0
BL1	<i>Pi1+Pi3</i>	8	89.3
BL2	<i>Pi1+Pi3</i>	7	90.7
BL5	<i>Pi1+Pi3</i>	5	93.3
BL3	<i>Pi2+Pi3</i>	4	94.7
BL4	<i>Pi2+Pi3</i>	5	93.3
BL6	<i>Pi1+Pi2+Pi3</i>	2	97.3
CO39 (ck)	None	75	0
Zhenshan 97	Unknown	46	38.7

Chen et al. 2001 Plant Disease



Hybrids and cultivars carrying these genes are at the stage of varietal trials and production demonstration.

GOAL 2: Reduction of fertilizer application

- Objectives seem well defined
- Researches are under way
- Prospects are clear

Reducing fertilizer application: technical definition

- Adequate uptake under field conditions of low nutrient concentrations (uptake efficiency)
- Efficient utilization of the absorbed nutrients (utilization efficiency)

Improving uptake efficiency

- Improving root architecture
- From passive to active absorption by enhancing transporter activity
 - High-affinity transporters (uptake under low concentration conditions)
 - Low-affinity transporters (uptake under high concentration conditions)

Improving the utilization efficiency: the N case

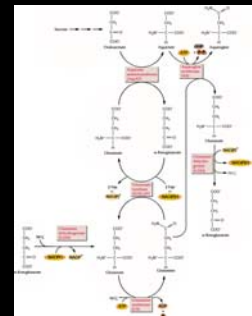
- Goals
 - Improving the efficiency of use and re-use
 - Reducing the loss in recycling
- Approaches
 - Modifying the genes involved in N assimilation pathways to improve the utilization efficiency
 - Discovering and identification of genes useful for improving NUE

Ammonium transporters in rice

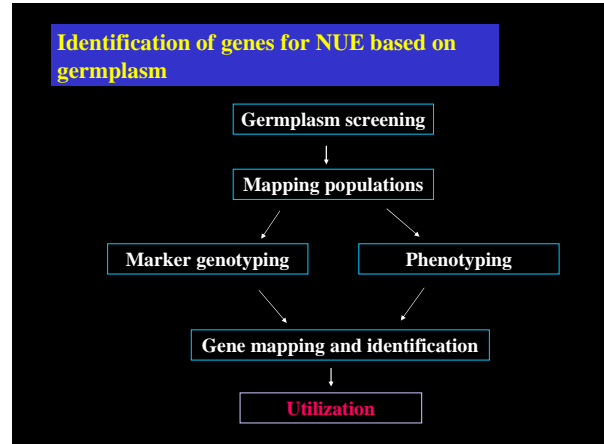
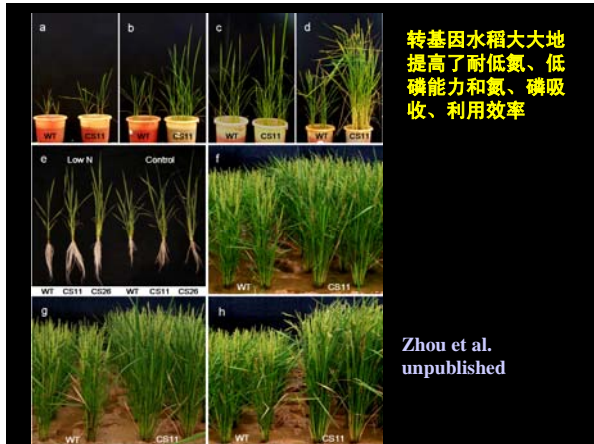
- *OsAMT1-1* AF289477/AAL05612 532aa
- *OsAMT1-2* AF289478/AAL05613 497aa
- *OsAMT1-3* AF289479/AAL05614 495aa
- *OsAMT2-1* AB051864/BAB87832 486aa
- *OsAMT2-2* AB083582/BAC65232 498aa
- *OsAMT2-3* NM_190448/NP_915337 500aa
- *OsAMT3-1* NM_190790/NP_915679 498aa
- *OsAMT3-2* XM_469225/XP_469225 353aa
- *OsAMT3-3* XM_469749/XP_469749 299aa
- *OsAMT4* XM_470358/XP_470358 479aa

Genes involved in N-assimilation pathways

- Ammonium assimilation
 - Glutamine synthetase (GS1, GS2)
 - Glutamate synthase (NADH-GOGAT, Fd-GOGAT)
 - Glutamate dehydrogenase (GDH)
- Ammonium transport and transfer
 - Aspartate aminotransferase (AspAT)
 - Asparagine synthetase (AS)



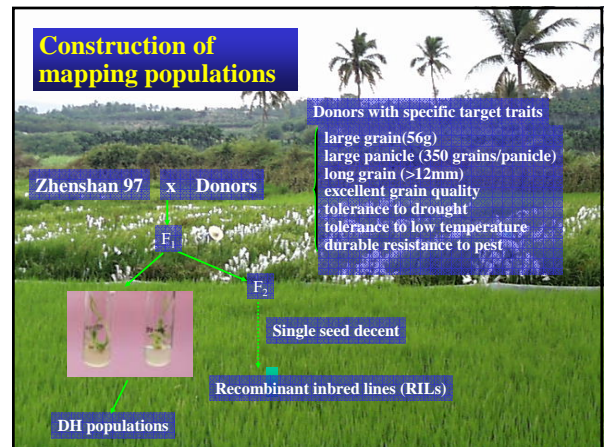
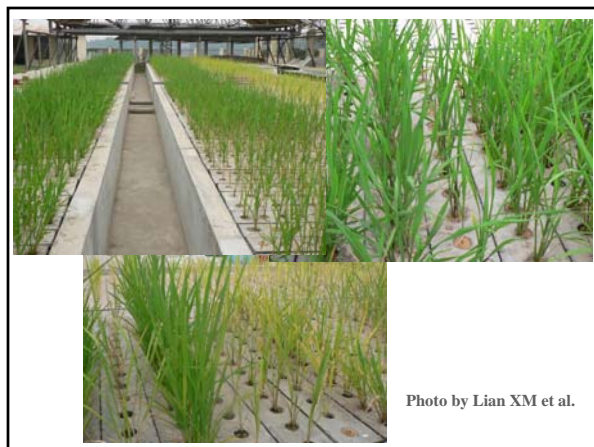
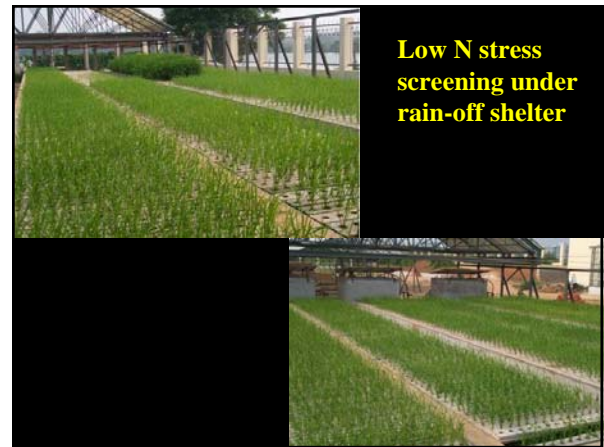
From Buchana et al. 2000

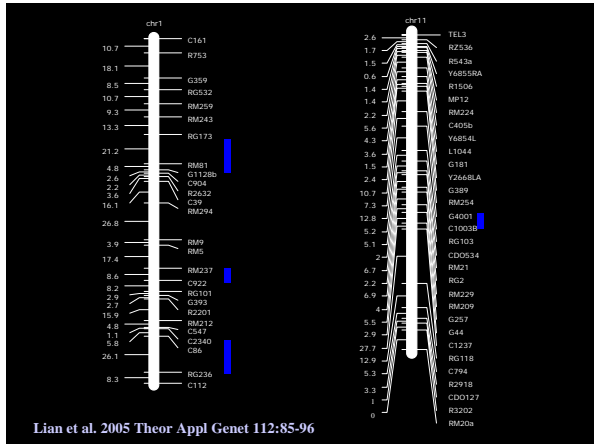


Core collections constructed by using diversity of whole genome SSR markers as the criteria

	Accs. in the bank	Core collection		Mimi-core collection		
		Acc.	% No. diversity	Acc.	% No.	% diversity
Rice	61479	3074	5 89.9	300	0.5	66.6
Wheat	23135	1160	5 90.1	231	1.0	69.1
Soybean	28809	1439	5 91.0	280	1.0	71.0

Data source: Prof. Jia Jizeng (2004)

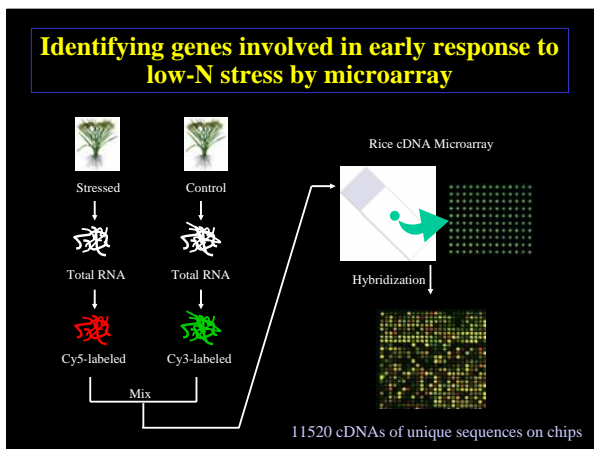
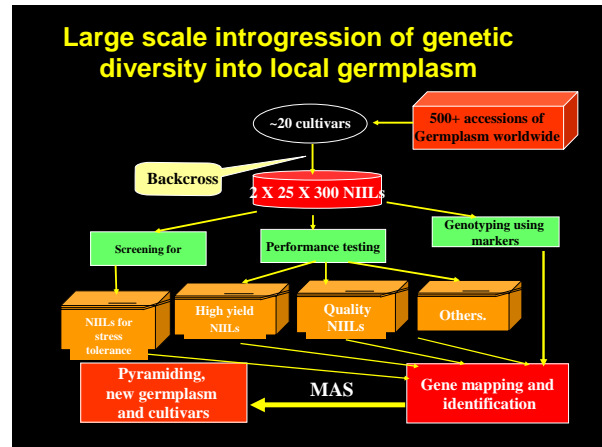
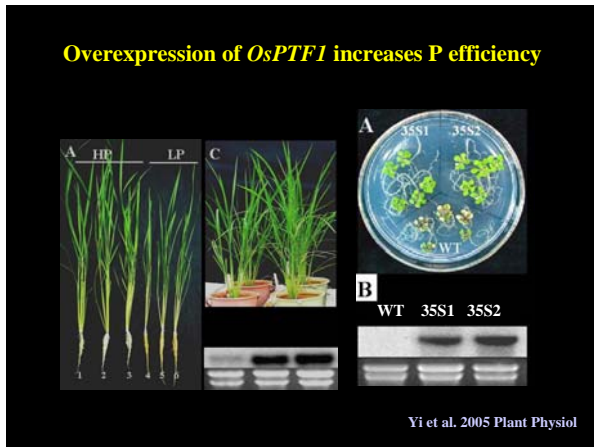




QTLs identified for low N tolerance using Zhenshan97/Minghui 63 RIL population

QTL	Exp	Chrom	Interval	LOD	Var (%)	A
NUE1	1	1	RG173-G1128b	3.16	5.8	0.020
NUE1	2	1	RG173-G1128b	2.67	4.1	0.017
NUE2	1	1	RM237-C922	4.07	7.8	-0.024
NUE2	2	1	RM237-C922	5.53	9.9	-0.027
NUE3	1	1	C86-RG236	3.08	5.6	-0.020
NUE3	2	1	C86-RG236	4.15	6.4	-0.021
NUE4	1	11	G4001-C1003B	2.78	5.6	-0.020
NUE4	2	11	G4001-C1003B	4.03	7.5	-0.023
NUE5	1	3	Rg393-C1087	2.65	4.0	0.016

Lian et al. Theor Appl Genet 2005



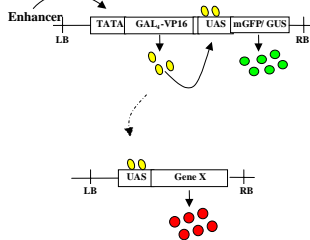
Genes selected from the chip results for functional testing

GENE	Ratio(T/C)	Function
CHIP17-C24	4.8	Ran-binding protein
CHIP18-O1	5.3	Hypothetical protein
CHIP19-I06	2.3	expressed protein
CHIP20-H17	6.4	Centromere protein
CHIP20-K18	3.3	Putative carnitine/acylcarnitine protein or mitochondrial carrier protein family
CHIP22-H13	2.2	Unknown protein or Zinc finger protein
CHIP14-F01	0.26	33-Kda secretory protein / protein kinase-related protein
CHIP05-E09	0.35	H-transferring ATP synthase chain 9-like precursor
CHIP15-B08	0.26	Atraabp1b/Ran binding protein
CHIP19-H19	0.2	Unnamed protein
CHIP01A21	3.2	unknow
CHIP01G09	3.8	xyloglucan endotransglycosylase
CHIP01H10	6.5	lipid transfer protein
CHIP01I01	4.0	unknown
CHIP03K02	2.3	photosystem II oxygen-evolving complex protein 2 precursor
CHIP04A09	4.1	putative elicitor-inducible protein
CHIP07M18	3.9	putative resistance protein
CHIP08E09	2.2	putative ubiquinone/menaquinone biosynthesis methyltransferase

Utilization of the mutant library for discovering genes for NUE

- The mutant library has three built-in strategies for functional identification of the genes
- >270,000 independent transformants generated
- More than 20,000 flanking sequences currently isolated
- Mutant lines were screened for various traits

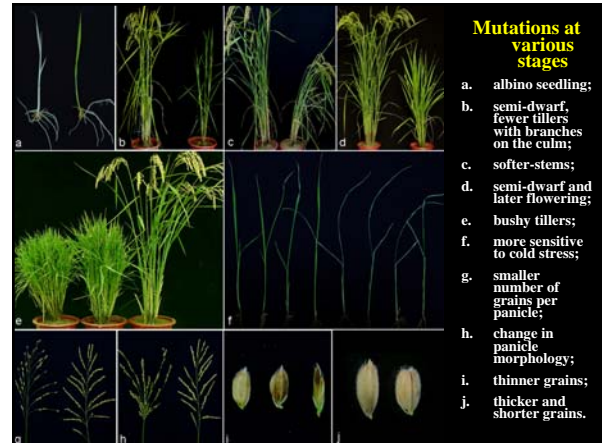
The working principle of the system



Three strategies for functional analysis of the rice genome

1. Insertional mutagenesis
2. Enhancer trapping
3. Ectopic expression

Wu et al. 2003 Plant J. 35:418-427



Mutations at various stages

- a. albino seedling;
- b. semi-dwarf, fewer tillers with branches on the culm;
- c. softer-stems;
- d. semi-dwarf and later flowering;
- e. bushy tillers;
- f. more sensitive to cold stress;
- g. smaller number of grains per panicle;
- h. change in panicle morphology;
- i. thinner grains;
- j. thicker and shorter grains.

T-DNA insertion mutant library screening



GOAL 3: drought resistance

- Urgent
- Complex
- Traits and targets have not been well defined

Improving drought resistance of irrigated rice

- Targeted stages: reproductive and maturity stages
- Mechanisms
 - Drought tolerance (shoot)
 - Drought avoidance (root)

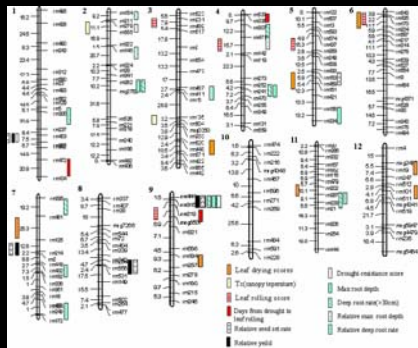
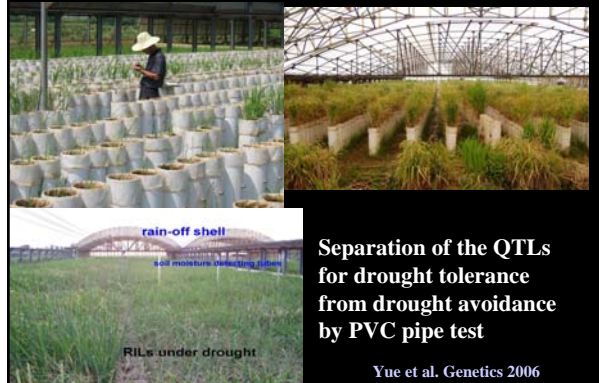
DR Phenotyping of the mini-core collection



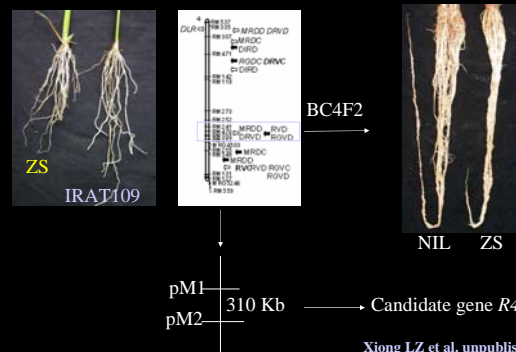
Mapping and identification of genes for drought resistance

- Mapping and breeding population
 - Zhenshan 97 (irrigated hybrid parent)/IRAT109 (upland)
 - 203 RILs, F7/F8

Drought stress of RILs for QTL mapping



Genetic improvement of root depth by QTL introgression



Transgenic rice of a candidate gene *R4* for *qMDR4-2* showed longer root than WT

R4 (IRAT109)
r4 (ZS97)

Deletion in the cDNA of *R4* in ZS97

Xiong LZ et al. unpublished



A drought tolerant line found in screening the T-DNA mutant library



Expression profiles of genes under drought stress

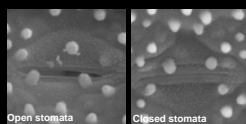
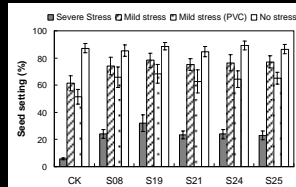
- **Materials**
 - Zhenshan 97: hybrid parent (irrigated rice)
 - IRAT109: upland rice

Drought responsive genes selected for transgenic analysis

Number of genes	Induction fold	Predicted function / Category	Gene family / description
11	1.6 – 7.2	Signal transduction	RLK, MAPK, CIPK, PP2C, ABA signaling, ...
22	1.6 – 5.2	Transcription factor	AP2, bZIP, ZF, NAC
4	1.7 – 4.1	Transportation	Transporter, antiporter, ...
12	2.9 – 5.4	Known function proteins	ABA synthesis, Protein for OA, anti-oxidation
8	1.7 – 10	Unknown function	--

Transgenic rice with enhanced drought resistance

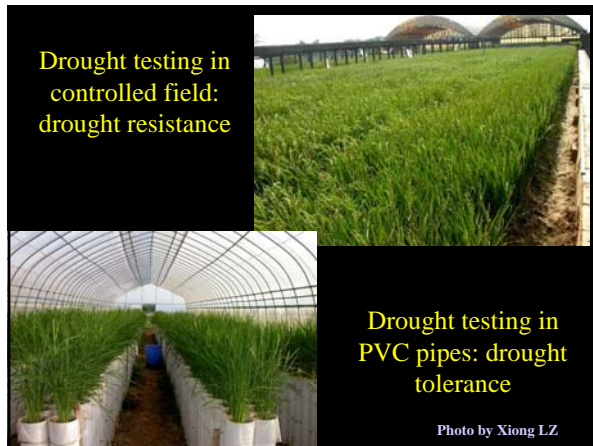
- Encoding a transcription factor
- Its over-expression enhanced resistance
- Regulating stomatal closure



Hu et al. PNAS 2006

Genes from various sources used for transforming rice for testing drought tolerance

Gene	Category	Gene product/function	Literature
CBF3	AP2 type transcription factor	Transcription factor	Jaglo-Ottosen et al., 1998; Kasuga et al., 1999
LOS5	ABA biosynthesis	Water status regulation	Xiong and Zhu, unpublished
SOS2	Protein kinase	Ion homeostasis	Guo and Zhu, unpublished
TPS	Trehalose-6-phosphate synthase	Novel osmoprotectant	Holmstrom et al., 1996; Yeo et al., 2000
HVA1	LEA protein gene	Tolerance to drought and salt stress	Xu et al. 1996
NPK1	MAPKKK	Detoxification of ROS	Kovtun et al., 2000
ZAT10	Zinc finger transcription factor	Transcription factor	Lee and Zhu, unpublished
NCED3	9-cis-epoxycarotenoid dioxygenase	Water status regulation	Qin and Zeevaert, 2002; Iuchi et al., 2001; Thompson et al., 2000
NHX1/2	Vacuolar Na ⁺ /H ⁺ antiporter	Ion homeostasis	Apse et al., 1999
CodA	Choline oxidase	Novel osmoprotectant	Sakamoto et al., 1998, 2000; Prasad et al., 2000



Grain yield of T1 transgenic families from each construct under normal and two drought stress conditions

Construct (promoter:gene)	Grain yield (g/plant) ^a			Relative yield ^b	
	Normal growth	Drought stress in field	Drought stress in PVC	Drought stress field	Drought stress field PVC
Wild type (ZH11)	32.55±1.98	7.64±0.23	21.11±0.92	0.23	0.65
<i>Actin1-CBF3</i>	17.65±0.89**	3.74±0.30*	13.76±0.62**	0.21	0.78**
<i>HVA22P-CBF3</i>	14.59±0.67**	4.80±0.25*	12.42±0.52**	0.33**	0.85**
<i>Actin1-SOS2</i>	17.70±0.89**	4.36±0.33*	12.32±0.61**	0.25	0.69
<i>HVA22P-SOS2</i>	15.91±0.81**	7.30±0.31	9.96±0.55**	0.46**	0.59
<i>Actin1-NCED1</i>	22.87±0.79*	4.89±0.28*	13.14±0.59**	0.21	0.57
<i>HVA22P-NCED1</i>	22.20±2.29*	3.98±0.28*	13.96±0.73**	0.18	0.63
<i>Actin1-NPK1</i>	18.50±1.07**	2.78±0.19**	14.73±0.60**	0.15*	0.81**
<i>HVA22P-NPK1</i>	17.42±1.10**	5.15±0.27	14.36±0.69**	0.30*	0.83**
<i>Actin1-LOSS</i>	16.79±0.66**	7.81±0.39	13.23±0.61**	0.47**	0.79**
<i>HVA22P-LOSS</i>	14.14±1.32**	4.83±0.34*	11.10±0.66**	0.34**	0.79**
<i>Actin1-ZAT10</i>	15.99±0.79**	6.62±0.39	11.92±0.54**	0.41**	0.75*
<i>HVA22P-ZAT10</i>	15.76±0.85**	6.08±0.25	12.77±0.51**	0.39**	0.81**
<i>Actin1-NHXI</i>	17.76±0.90**	6.75±0.28	12.57±0.56**	0.38**	0.71*
<i>HVA22P-NHXI</i>	13.05±1.01**	3.56±0.25*	9.97±0.49**	0.27*	0.76*

GS3, a major QTL for grain size

- Grain size: a trait both for yield (grain weight) and quality (grain length)
- Encodes a transmembrane protein
- A premature termination results in large grain

Fan et al. 2006 Theor Appl Genet

Ghd7, a major QTL for plant height, heading date and gain number

Xue et al. 2008 Nat Genet

S5, 籼粳不育/广亲和基因

- 籼粳杂种生物学优势很强, 但育性普遍较低
- S5是调控籼粳杂种育性的一个主要位点
- S5编码一个天冬氨酸蛋白酶
- 广亲和基因是一个丧失功能的突变

Chen et al 2008 PNAS

Combinations of genes and approaches for the development of green super rice

Green super rice

↑

Transgenics
MAS
Traditional breeding

←

Super rice

←

Traits to be improved:
Insect resistances
Disease resistances
Nutrient efficiency
Drought resistance
Quality
Yield

←

Genes from non-rice sources

←

Genes based on rice germplasm

←

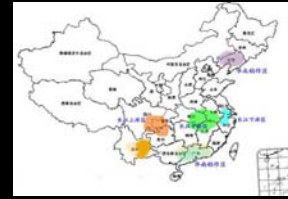
Genes based on functional genomics

Zhang Q 2007 PNAS 104:16402-16409

Perspectives

- Development of green rice is of paramount importance to the sustainability of agriculture, environment and human society.
- The task is multidisciplinary and requires collaborations of scientists working in different areas and countries.
- Arduous but no alternative.

Network of the Molecular Breeding Project



Participants:

Anhui Agric Acad
CAAS
CNRRRI
Guangdong Agric Acad
Huazhong Agric U
Jiangxi Agric Acad
Liaoning Agric Acad
Shanghai Agric Acad
Shanghai Agro G C
Shenyang Agric U
Sichuan Agric Acad
South China Agric U
Yunnan Agric Acad



Contributors

Groups in Huazhong Agricultural University
Plant nutrition (Xingming Lian)
Drought resistance (Lizhong Xiong)
Markers and mapping (Yongzhong Xing)
Transformation (Jumin Tu and Yongjun Lin)
Molecular breeding (Yuqing He)
Plant disease group (Shiping Wang)
Mutant library group (Changyin Wu)
College of Plant Science and Technology (Deming Jin)
College of Resource and Environment (Zhuqing Zhao)

Collaborators:

Shanghai Agro-Gene Center (Lijun Luo)
National Center of Gene Research, CAS (Bin Han)
Wuhan University (Guangcun He)
Chinese Academy of Agricultural Sciences (Yunliu Fan)
CAMBIA (A. Killian and R. Jefferson)
IRRI (S. Data, G. S. Khush)
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973

948

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The Rockefeller Foundation