



# **Rice Functional Genomics Programme in China**

**Hong-Wei Xue**

**National Key Laboratory of Plant Molecular Genetics  
Institute of Plant Physiology and Ecology, Chinese Academy  
of Sciences**

**20091106**

- **Agricultural demand**
- **Rice functional genomics program**

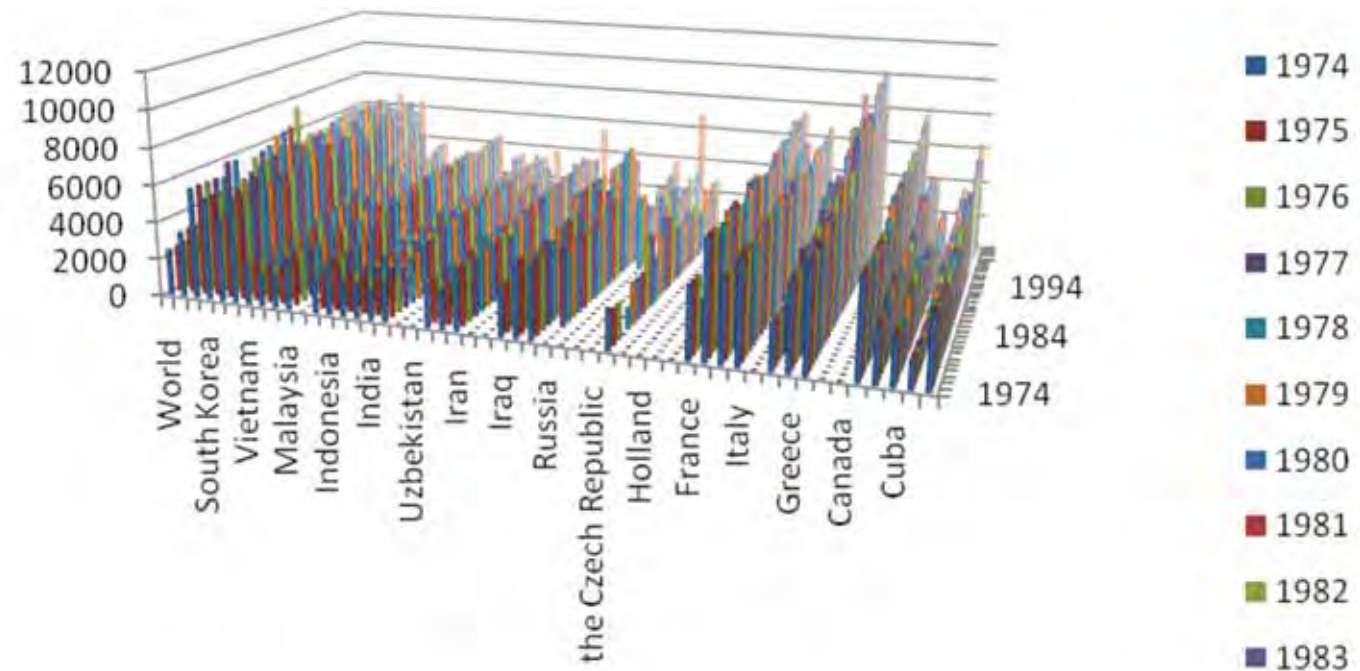
**Why rice**

**Progresses**

**How it helps the breeding**

- **Population and Land Situation of China:**  
**China breeds 22% population (1.3 billion)**  
**with 9% of the world's arable land in the last**  
**decades**
- **You can't build peace on empty stomachs.**  
**(Norman Borlaug, 1970 Nobel Peace Prize)**

- Rice is the most important food crop in the world;
- China is the largest rice producing country (~1/3 of the world total rice production);
- By green revolution and conventional breeding, the rice yield keep increasing, however, not as fast as the population.



Rice yield (Kg/Ha), from Wang DC, LP High-Tec.

**Three-hybrid rice research is initiated by Prof. Yuan Long-ping in China in 1964 and has taken the leading position in the world since then. Two-line hybrid rice research has been started since 1974's and succeed in 1995's, and has achieved a vital advances presently.**

**The cultivated area takes about 15% of the whole national hybrid rice planting area of China, reaches to 0.78 million ha in 2000's, 1.19 million ha in 2004's, and reached 2.16 million ha in 2006's (by statistics from National Agriculture Technical Extension).**

**From Wang DC, LP High-Tec.**

# Control of rice blast by molecular marker and genetic diversity



2001-2005, 2.2 million ha.rice field has used this technique in Yunnan and Sichuan et al., and was introduced into Philippine, Indonesia, Vietnam, Nepal and so on.

- **Higher yield**
- **Improved quality**
- **Stress tolerance / disease resistance**
- **Functional food (metabolites, altered nutrients, etc)**
- **Bioreactors (vaccine, etc)**

**Expand the genetic diversity of current breeding lines**

# The main current breeding methods

- **Conventional breeding** (selective breeding, sexual hybrid breeding, heterosis breeding, physical and chemistry mutation breeding, *in vitro* breeding, etc)
- **Molecular Breeding (Marker Assisted Selection, transgenic plants)**
- **Distant hybridization technology: by hybridization of rice with maize, sorghum or other varieties in suntan relative genetic distance to enrich the gene bank of rice, and enlarge the genetic background of rice, which may result in the huge increase of yield.**

- **Identified many resources/markers related to important agronomic traits**
- **Marker-assisted selection (MAS)**
- **Systemic studies on rice development**
- **China rice functional genomics program is initiated since 1999 (Chinese Academy of Sciences, Ministry of Science and Technology, MOA)**
- **Focusing on large scale resources; development of plant types; fertility control; seed development and endosperm development; heterosis; sugar metabolism; stress responses; disease resistance, etc.**

- **Agricultural demand**
- **Rice functional genomics program**

**Why rice**

**Progresses**

**How it helps the breeding**

- **Rice is one of the most important crops in the world;**
- **China is the largest rice producing country, also consuming one;**
- **The completion of the genome sequencing project.**
- **Rice is becoming the model for genome research of cereals----on barley, wheat, maize, brassica species, others.**

# First phase (2002-2005)

- **Development of technological platforms**
  - Mutant population
  - Microarray and expression profiling
  - Full-length cDNA
  - Data integrations
- **Functional genomics of agriculturally important traits**

Disease resistance (blast and bacterial blight); Drought resistance; N- and P-use efficiency; Plant growth and development; Root pattern; Grain quality; Yield and heterosis
- **Molecular breeding**

- **Development of technological platforms**

**Aimed at high through analyses and effective characterization of gene functions**

- **Generation and characterization of a large mutant library by T-DNA insertion**
- **Global expression profiling of genes in the entire genome**
- **Isolation of full-length cDNAs**

- **Totally 270,000 independent transformants, generated for the T-DNA insertion mutant (knockout, gain-of-function) library (Wuhan, Beijing, Shanghai)**
- **Over 40,000 flanking sequences have been isolated, a number of interesting features of nonrandom distributions of the T-DNA insertions have been identified in the rice genome**



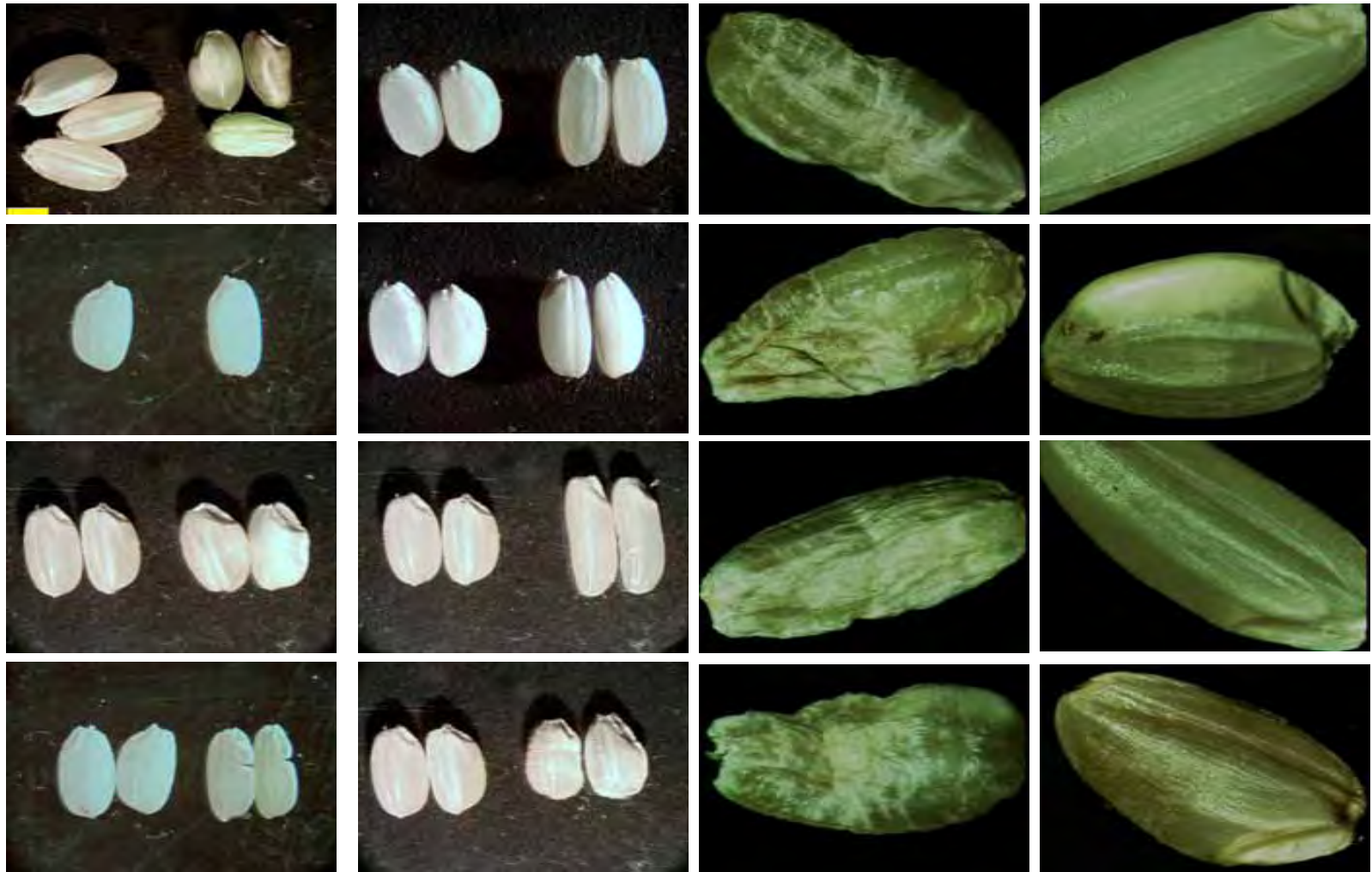
## Flowering time



## Altered panicle development

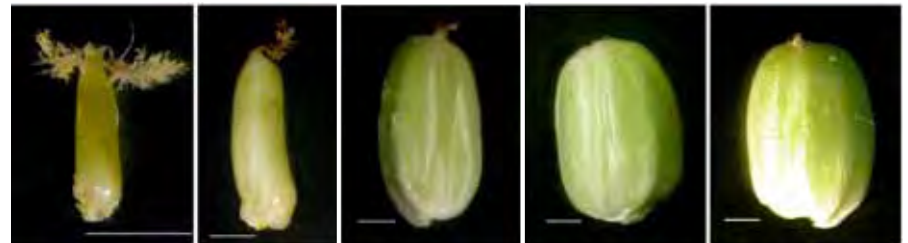


# Abnormal seed shape



- **For genome-wide expression profiling, data collected from more than 40 tissues covering the whole life cycle of the rice plants and under various conditions.**

**Candidate genes involved in various developmental processes or response to environmental stimuli.**



- **Functional genomics of agriculturally important traits**

**Yields, grain filling**

**Tiller control (number and angle)**

**Stresses (disease resistance - blast and bacterial blight; drought resistance; salt tolerance)**

**Hybrid rice, heterosis**

***Ghd7* and *GW2* are important regulators controlling rice grain width and weight**



**(Nature Biotech, 2007, Nature Genetics, 2007)**

- **GS3 (at Chr.3, controls grain length and weight)**
- **GW2 (at Chr.2, contributes 65.5% of grain width)**
- **Ghd7 (plant height, heading date, grain number per panicle)**
- **DEP1 (panicle morphology)**
- **GIF 1 (grain filling)**
- **EUI (controls the growth of upmost internode)**

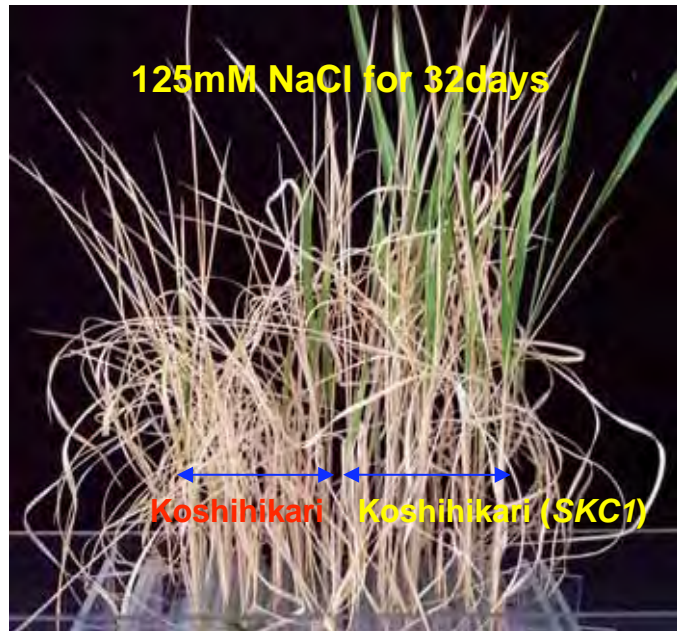
**MOC1 controls tiller numbers**

**LAZY1, LHD2, TAC1, PROG1 control tiller angles**



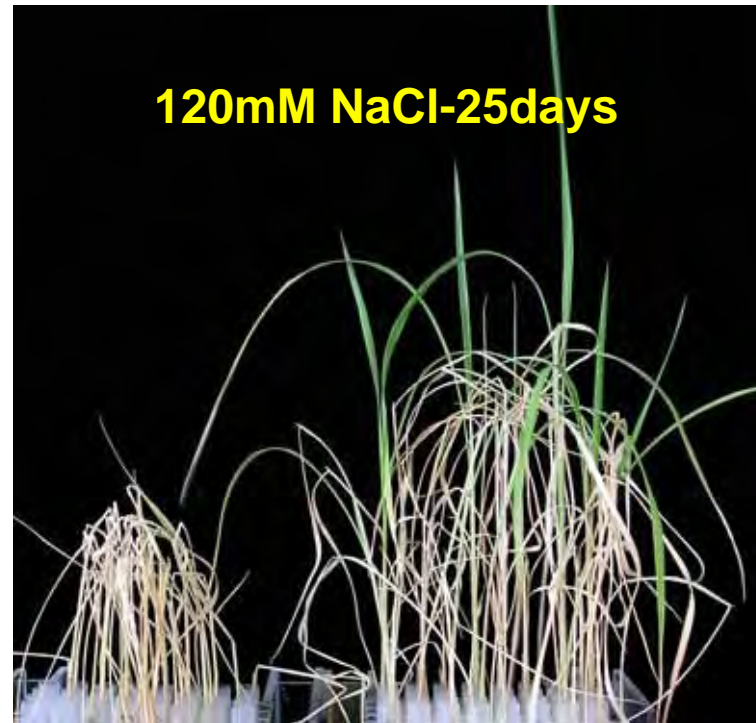
**Li et al., Nature, 2003; Cell Res, 2006**

- **Disease resistance: Xa3/Xa2b, Xa13, Pigm**
- **Salt tolerance: SKC1, OsHAL3**
- **Draught tolerance: SNAC1, OsSKIPa**



*SKC1* is available in marker-assistant breeding for improving rice salt tolerance

Ren *et al.*, *Nature Genetics*, 2005



**Vector**

**OE-HAL3  
(overexpression lines)**

*Sun et al. Nature Cell Biology, 2009*

## Genes for hybrid rice

- **WA CMS (orf52)**
- **Fertility restorer genes**
- **S5, wide compatibility gene (Chen et al., 2008)**
- **Sa, hybrid sterility gene (Long et al., 2008)**

- **Molecular breeding**

**how it help to counter the narrow  
genetic diversity of current breeding  
lines**

**Quality**

**Yield**

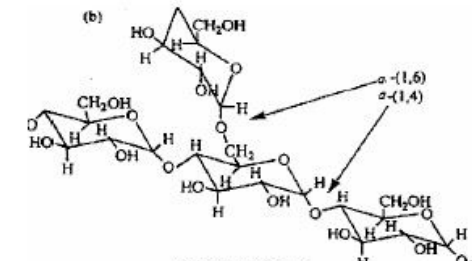
**Architecture, tillers, leaf**

**Hybrid rice**

**Stress tolerance, disease resistance**

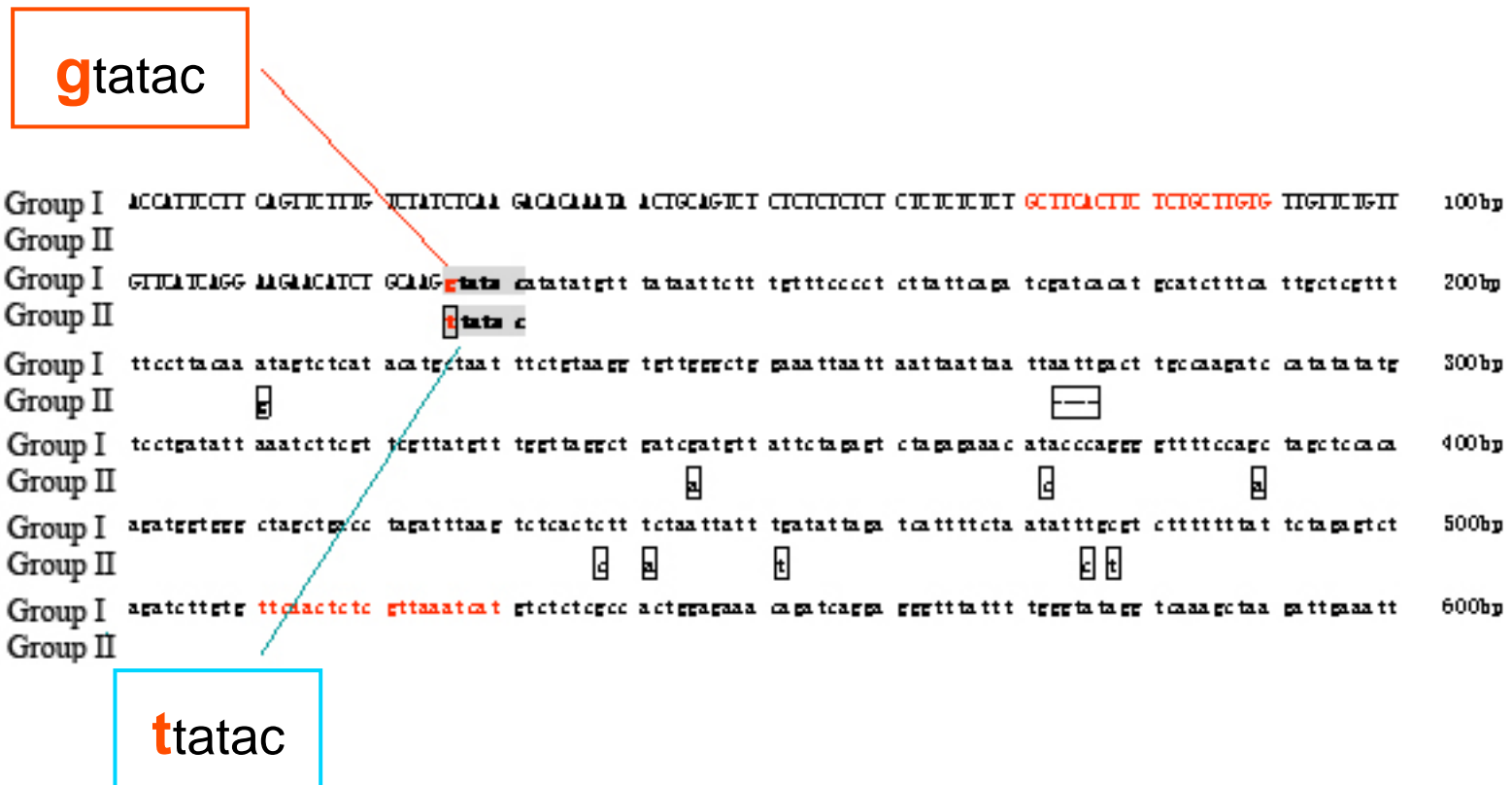
# Starch: main composition of rice endosperm (77%~78%)

- **Amylose**  
indica rice, 20%~30%; japonic rice, 15%~22%.
- **Amylopectin**  
70% ~80% of total starch
- **Cooking and eating quality**
- **High amylose in the endosperm is usually associated with dry, fluffy, and separated cooked rice grain, and represents the key determinants of poor cooking and eating quality.**
- **The rice waxy (*Wx*) gene encodes for the starch granule-bound starch synthase (EC2.4.1.11), and is responsible for the synthesis of amylose in the endosperm and pollen.**



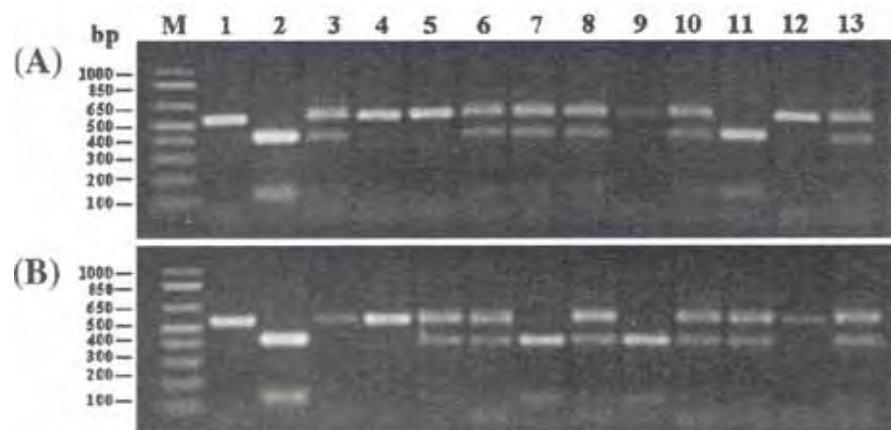


A CAPS marker (Named as PCR-Accl, Patent No. ZL00116700.6) was developed based on the G→T mutation at the +1 splicing donor site of Wx intron 1



In the cultivars with high amylose (group I), the nucleotide at +1 splicing donor site is G, a Accl recognition site “gtatac” is occurred. While in the cultivars with intermediate amylose (group II), the nucleotide at +1 splicing donor site is changed from G to T and the Accl recognition site is therefore abolished.

## Improving cooking and eating quality of elite parents of Hybrid Rice by using the molecular marker-assisted selection



Determination of the PCR-Accl molecular marker in individuals of the BC<sub>4</sub>F<sub>2</sub> generation from two target parents

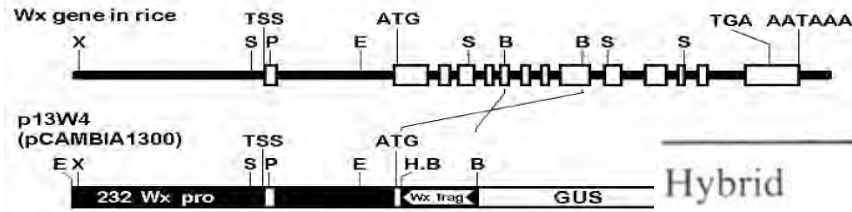
| Hybrid           | Amylose content (%) | GC (mm) | GT (ASV) | Peak viscosity (RVU) | Hot viscosity (RVU) | Breakdown (RVU) | Cool viscosity (RVU) | Setback (RVU) | Consistence (RVU) | Peak time (min) | Pasting temperature (°C) |
|------------------|---------------------|---------|----------|----------------------|---------------------|-----------------|----------------------|---------------|-------------------|-----------------|--------------------------|
| Teyou 63         | 22.5                | 34.8    | 5.4      | 2882                 | 2180                | 702             | 3544                 | 662           | 1364              | 6.53            | 74.5                     |
| LTF(tt)-A-1/MH63 | 16.1**              | 82.5**  | 3.7*     | 2904                 | 1648**              | 1256**          | 2662**               | -242**        | 1014*             | 6.20            | 74.4                     |
| Shanyou 63       | 21.4                | 61.0    | 4.5      | 2983                 | 1641                | 1342            | 3033                 | 50            | 1392              | 5.80            | 80.0                     |
| ZS(tt)-A-1/MH63  | 16.0**              | 82.0*   | 2.4*     | 2965                 | 1460*               | 1505*           | 2437**               | -528**        | 977*              | 5.93            | 79.9                     |

\* Significant at the  $P = 0.05$  probability level when compared with the original parent.

\*\* Significant at the  $P = 0.01$  probability level when compared with the original parent.

The AC, GC, GT, and starch pasting properties (starch viscosity characteristics) of the improved hybrid rice under regular field conditions

# Improving cooking and eating quality in transgenic *indica* Hybrid Rice by down-regulation of *Wx* gene expression



| Hybrid     | AC (%)  | GC (cm) | GT (ASV) |
|------------|---------|---------|----------|
| year 2002  |         |         |          |
| Teyou 63   | 24.12   | 5.60    | 4.83     |
| L25A/MH63  | 12.13** | 10.45** | 4.12*    |
| L18A/MH63  | 20.56** | 6.10*   | 4.70     |
| year 2003  |         |         |          |
| Teyou 63   | 24.23   | 5.00    | 4.92     |
| L25A/MH63  | 11.09** | 9.95**  | 4.17*    |
| L18A/MH63  | 22.14*  | 5.45    | 4.79     |
| Teyou 559  | 24.28   | 4.67    | 3.71     |
| L25A/YH559 | 10.06** | 10.80** | 2.77*    |
| L18A/YH559 | 21.16** | 5.05*   | 3.54     |

\* and \*\* the least significant differences at 0.05 and 0.01 probability levels, respectively, when compared with the original parent; no label means no significant difference.

## **GW2, a QTL for grain width and weight control**



**For yield increase of rice of south regions**



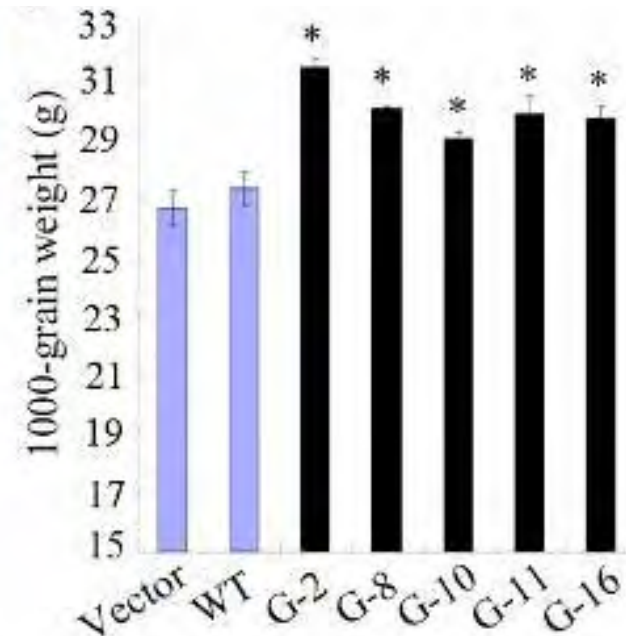
**Control**

**AS3**

**AS24**

**Song et al., Nature Genetics, 2007**

## Enhanced expression of *GIF1* increases grain size and weight of rice



It documents that modern varieties could be further improved with a domestication gene

# ***Moc1*: a gene controlling tillering**



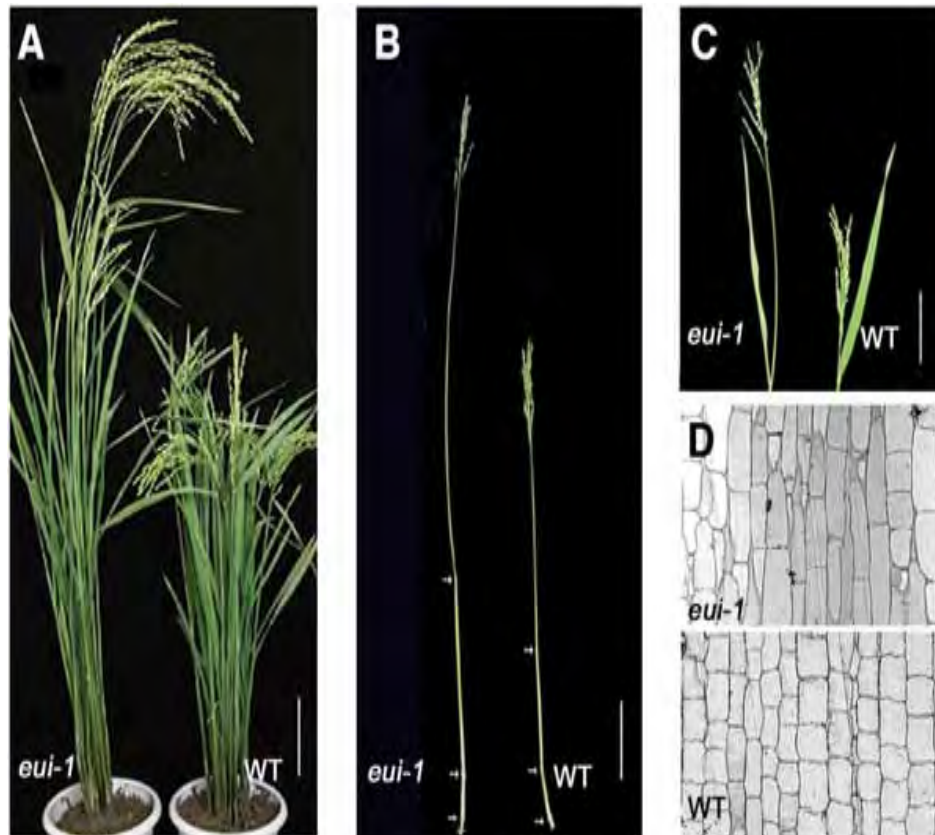
*Moc1* encodes a putative GRAS family nuclear protein expressed mainly in the axillary buds functions to initiate axillary buds and promote their outgrowth



**For architecture  
improvement of super-rice**

**Li et al., Nature, 2003**

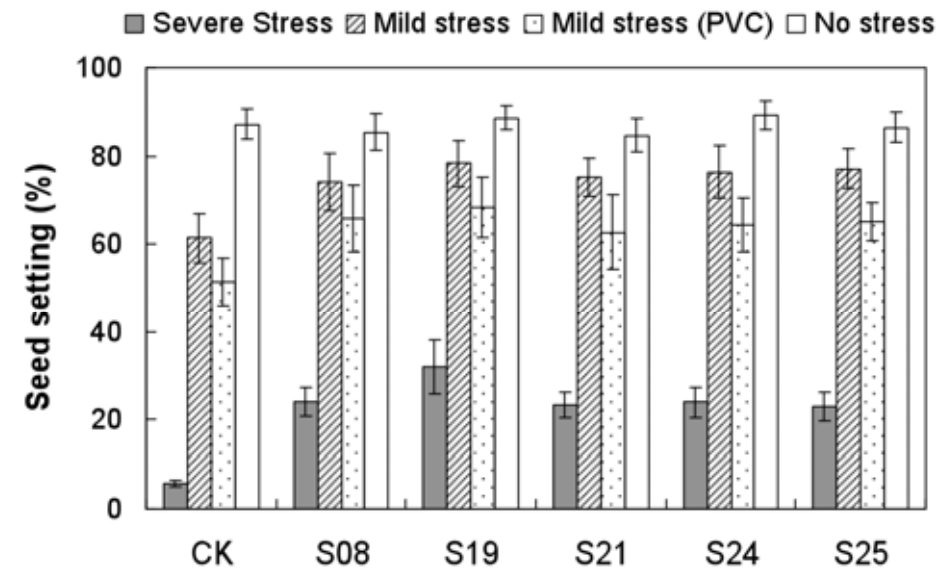
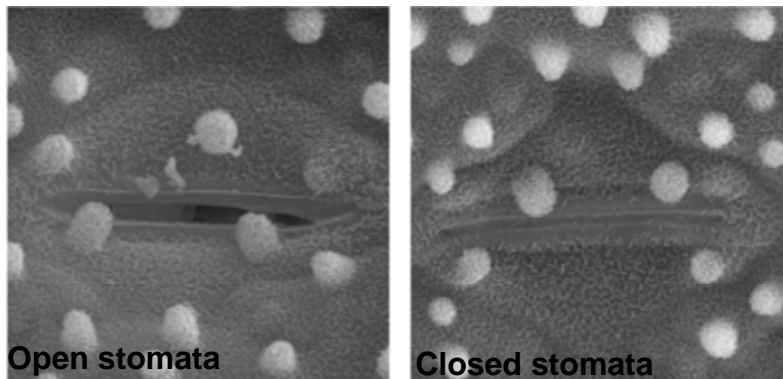
***ELONGATED UPPERMOST INTERNODE* encodes a  
Cytochrome P450 monooxygenase that epoxidizes  
gibberellins**



The *eui* mutants has been used in hybrid rice breeding to eliminate panicle enclosure in male sterile lines to develop **e-rice**, which greatly decreases gibberellin use and increases yield and quality of hybrid seed production. (from He Zuhua, SIPPE)

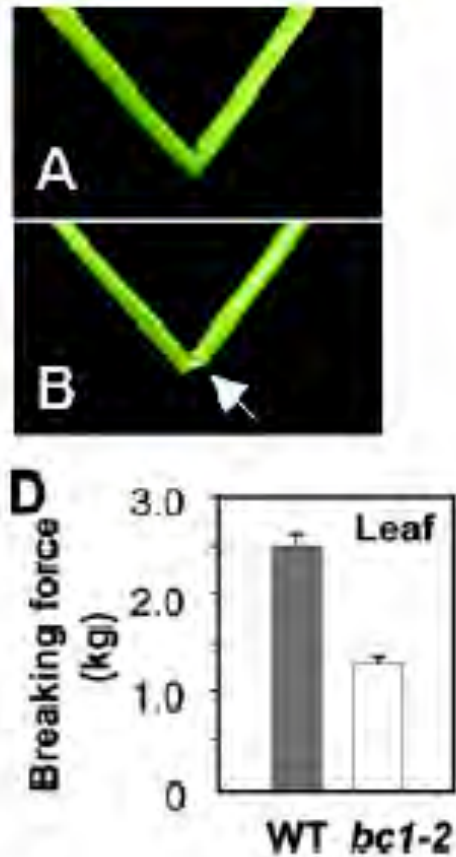
# Transgenic rice with enhanced drought resistance

- A transcription factor
- Over-expression enhanced resistance
- Regulating stomatal closure



Hu et al., PNAS, 2006,12992

## *Bc1*: a gene for brittle culm



*For rice breeding of straw of those will be used as feed*

**Yield controlling gene: GS3 (grain length and weight)**

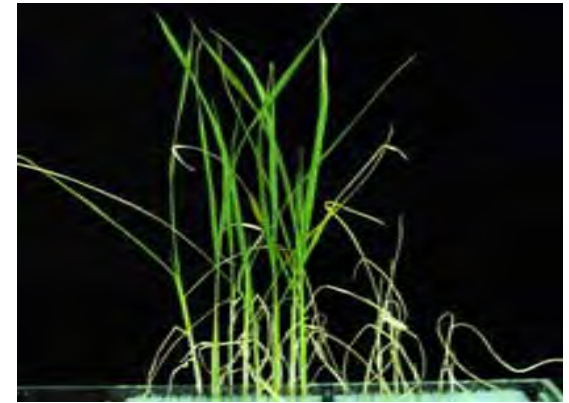
**Grain filling controlling gene: GIF1**

**Heading date controlling gene: Ghd7**

**Salt tolerance gene: SKC1**

**Blast resistance gene: PIGM**

**Disease resistance genes: Xa3/Xa2b, Xa13**



## **Second phase (2006-2010)**

- **Further development of technological platforms**
- **Functional genomics of more agriculturally important traits – seed/endosperm**
- **Coordinated phenotyping of mutations**
- **Develop new technologies for breeding**
- **Core germplasm – resequencing– important traits**

## Increasing demand

- **Challenges:** increasing population, decreasing farm land, environmental impact, etc
- **Yield, quality, resistance, expanded genetic diversity of current breeding lines**

**More resources and supports: genetics materials, technologies (high-throughput), funds (government, company)**

**Provide more genes/markers**

**Develop new technologies**

**(RNi, marker-free, non-BT, etc)**



# Acknowledgements

Collaborators (materials, informations, comments)

