

Research and Applications of Honglian Hybrid Rice

Yingguo Zhu

College of Life Science, Wuhan University

Contents

✦ **Development of Honglian hybrid rice**

✦ **Novel germplasm development**

✦ **Mechanism of Honglian CMS**

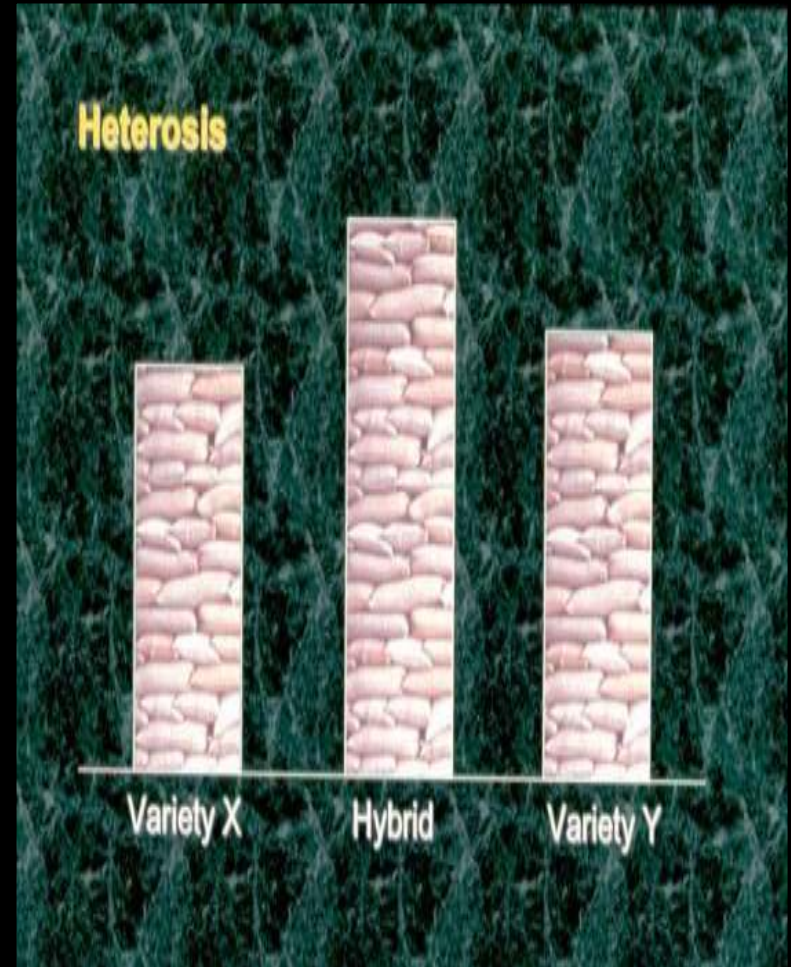
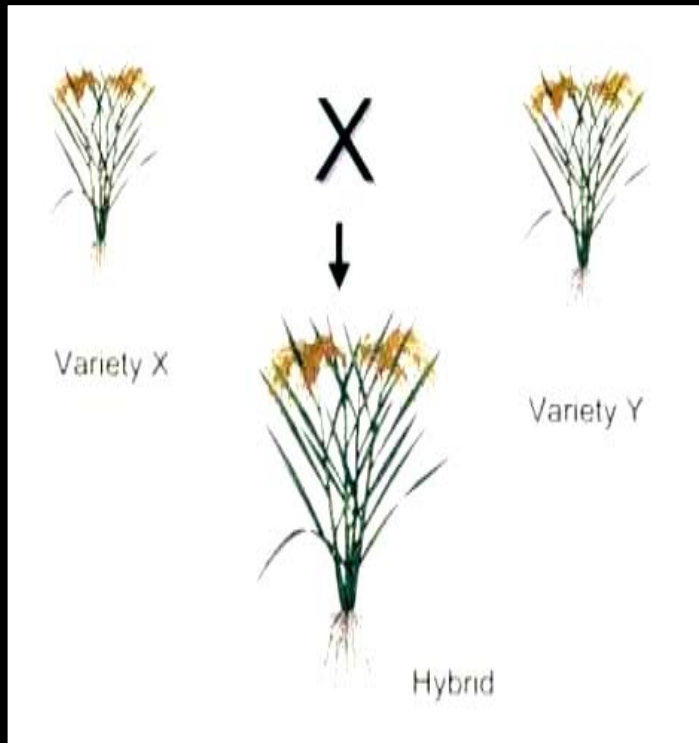
The food security



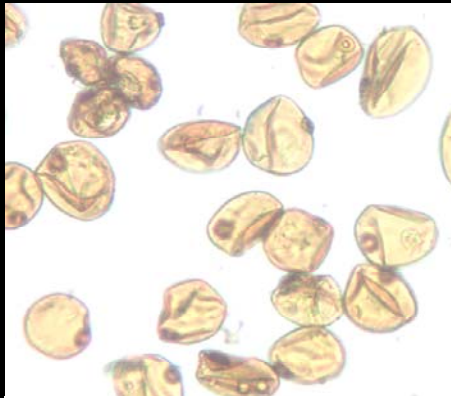
Rice demand

- * Rice demand rise by 40% by 2025, due to population increase and improved incomes
- * agricultural land will not increase
- * Need to develop and use technologies that lead to more rice per unit area/time ,low the unit price

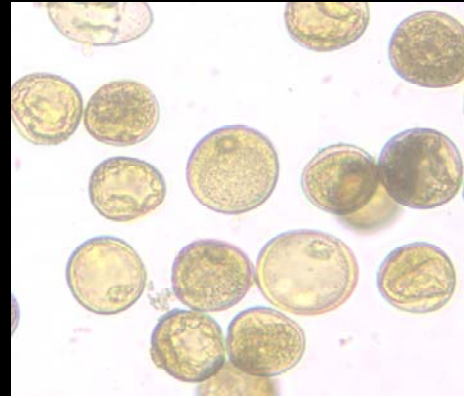
Hybrid rice is an effective approach to increase rice grain yield



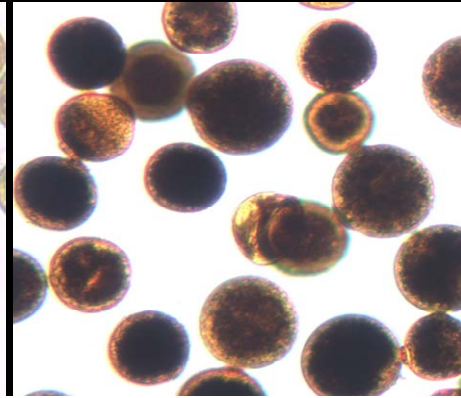
Honglian CMS is one of the three main CMS types used in China



Wild-abortive



Honglian



Boro

Name of CMS	CMS type	Abortive stage	Fertility Restorer
Wild abortive CMS	Sporophytic	Mononucleate stage	Rf3/Rf4
Honglian CMS	Gametophytic	Dinucleate stage	Rf5/Rf6
Boro CMS	Gametophytic	Trinucleate stage	Rf1(PPR)

- The three main CMS types in the world: Wild abortive, Honglian , Boro
- The fertility restoration pattern of HL is similar to Boro, but different from WA type

The discovery of HL CMS rice

O. Rufipogon (Red awn)/LiantangZao

Hong-lian A (1974)

Hua-ai 15A (1976)

YuetaiA

LuoHong 3A

Honglian you-6
Yueyou-9

LuoYou 8





Development of Honglian Hybrid Rice

Scale up of fundamental seeds of HL-CMS for hybrid rice seed production

a standard operation protocol (SOP)



Core seeds



Original seeds



Primary seeds



Fundamental seeds

Keep stable sterility of HL-CMS line

Fertility observation and purity identification of HL-CMS line Yuetai-A

site	date	Population size	Abnormal plant	Sterile plant ratio(%)	Checked spikelet	Sterile pollen(%)	Bagged plant	Bagged set rate(%)
Hainan lingshui	2000.11.10	13900	0	100	228	99.98	112	0
Hubei Wuhan	2001.07.20	1510	0	100	206	99.95	110	0
Hainan lingshui	2004.11.28	14353	52	99.64	100	99.99	120	0.01
Hubei Wuhan	2005.7.30	1148	4	99.65	101	99.83	115	0.02

Fertility observation showed that in a 1000-plant population, the sterile pollen over **99.95%**; sterile spikelet over **99.98%**





2002 8 4

The first variety Honglian You-6 was certificated in Hubei Province, in 2002



Honglian You-6 was certificated in 2004 by Chinese National Crop Variety Approval Committee (CNCVAC)

Honglian You-6 was honored 1st Award of Hubei Science and Technology Progression in 2004



Total of more than
one million hectares
have been cultivated
in China since 2002

Novel varieties of Honglian hybrid rice

湖北省农作物品种审定 证书

审定编号：鄂审稻 2006005
作物种类：水稻
品种名称：珞优 8 号
选育单位：武汉大学生命科学学院

证书编号：466

品种名称：珞优 8 号
品种来源：武汉大学生命科学学院用不育系“珞红 3A”与恢复系“8108”配组育成的杂交中稻品种。区试代号：红莲优 8 号。2006 年通过湖北省农作物品种审定委员会审定，品种审定编号为鄂审稻 2006005。

品质产量：2004~2005 年参加湖北省中稻品种区域试验，米质经农业部食品质量监督检验测试中心测定，出糙率 80.9%，整精米率 62.8%，垩白粒率 19%，垩白度 1.9%，直链淀粉含量 21.78%，胶稠度 56 毫米，长宽比 3.2，主要理化指标达到国标二级优质稻谷质量标准。两年区域试验平均亩产 566.08 公斤，比对照 II 优 725 增产 0.77%。

特征特性：株型紧凑，株高适中，茎节部分外露，茎秆韧性较好，叶色浓绿，剑叶较窄长、挺直，叶鞘无色。穗层整齐，谷粒长型，稃尖无色，部分谷粒有短顶芒。有两段灌浆现象，遇低温有包颈和麻壳，后期转色一般。区域试验中亩有效穗 17.9 万，株高 120.7 厘米，穗长 23.5 厘米，每穗总粒数 161.6 粒，实粒数 125.1 粒，结实率 77.4%，千粒重 26.83 克。全生育期 141.7 天，比 II 优 725 长 2.0 天。抗病性鉴定为高感穗颈稻瘟病，感白叶枯病。田间稻曲病较重。

适宜范围：适于湖北省鄂西南山区以外地区作中稻种植。



Luoyou-8:

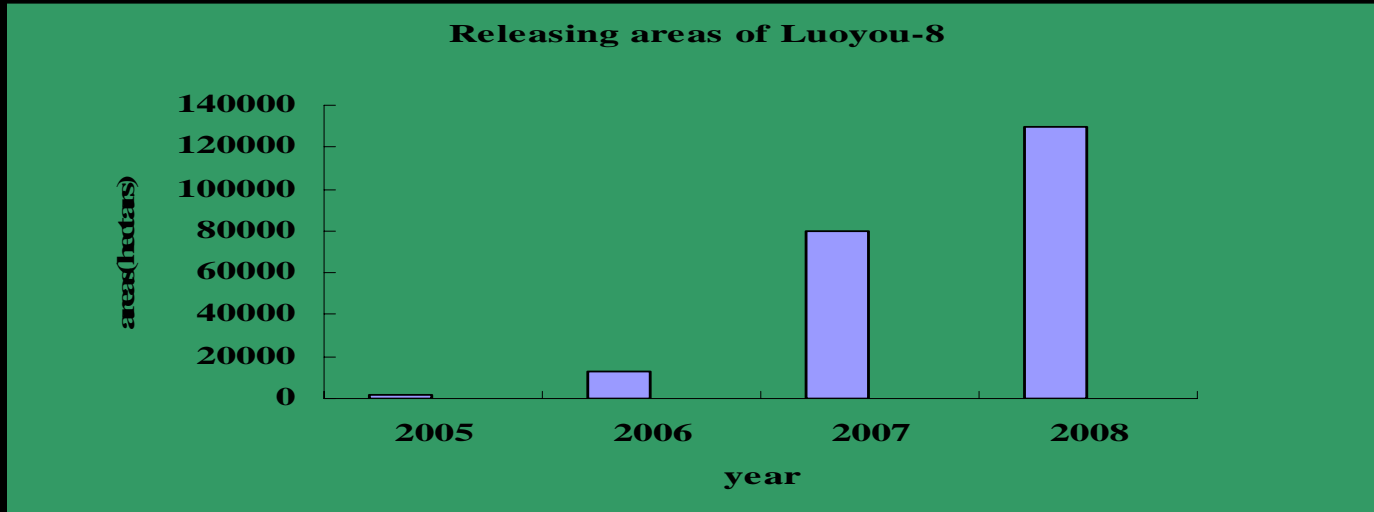
Approved by Hubei province crop variety approval committee (2006)

Approved by Chinese National crop variety approval committee (2007)

Yueyou-9:

Approved by Hubei province crop variety Approval committee (2006)

Luoyou-8 is swiftly popularization in the Yangtze valley during 2005-2008



Characteristic of Honglian Hybrid rice

- ✦ **Higher grain yield**
- ✦ **Higher grain quality**
- ✦ **Higher Nitrogen utilization efficiency**
- ✦ **Better performance characters**
- ✦ **Wider adaptability**
- ✦ **Easy for hybrid-seed production**
- ✦ **Higher resistance to high temperature**

Better eating quality



All HL-type hybrid rice had been recognized to meet the criteria of Chinese high eating quality



Honglian you-6

- Honglian you-6 qualified the criteria of the third grade grain ;
- Luoyou-8 qualified the criteria of the second grade grain



**Higher grain
yield potential**

Honglian you-6 increase 6.17% over elite variety at the field trials in Hubei Province, Grain yield potential reached to **12 tons/hectare**

红莲型杂交稻“珞优8号”现场验收意见

2007年9月17日，湖北省科技厅组织国内有关专家，对武汉大学选育的红莲型杂交稻“珞优8号”在湖北省农业科技创新中心试验示范现场，进行了测产验收，专家组采用理论测产和实收相结合的方法进行，综合意见如下：

- 1、珞优8号在试验示范基地种植18亩，田间生长整齐一致，纯度高，株形较紧凑，后期落色好，米质优。
- 2、试验基地种植的珞优8号于2007年5月7日播种，6月2日移栽，8月13日齐穗，9月15日成熟，全生育期132天。
- 3、理论测产：随机取样调查，每亩插植1.52万蔸，每亩有效穗16.9万，每穗总粒数204.4，每穗实粒178.9，结实率87.5%，千粒重按27g计算，每亩理论产量816.3kg。
- 4、选择具有代表性的田块机械收割，面积999.1m²，收获净稻谷1321.1kg，含水量20.1%，按含水量13.5%计算，每亩稻谷产量823.4kg。

组长：

陈云

副组长：

李林

2007年9月17日

2007

Luoyou-8 showed higher yield potential in Wuhan field trial, reached 12.35 tons/hectare.

Wider adaptability

- South-eastern Asia
- Yangtze valley in China
- South-western region of China
- Southern region of China

水稻集中产区

水稻分散产区

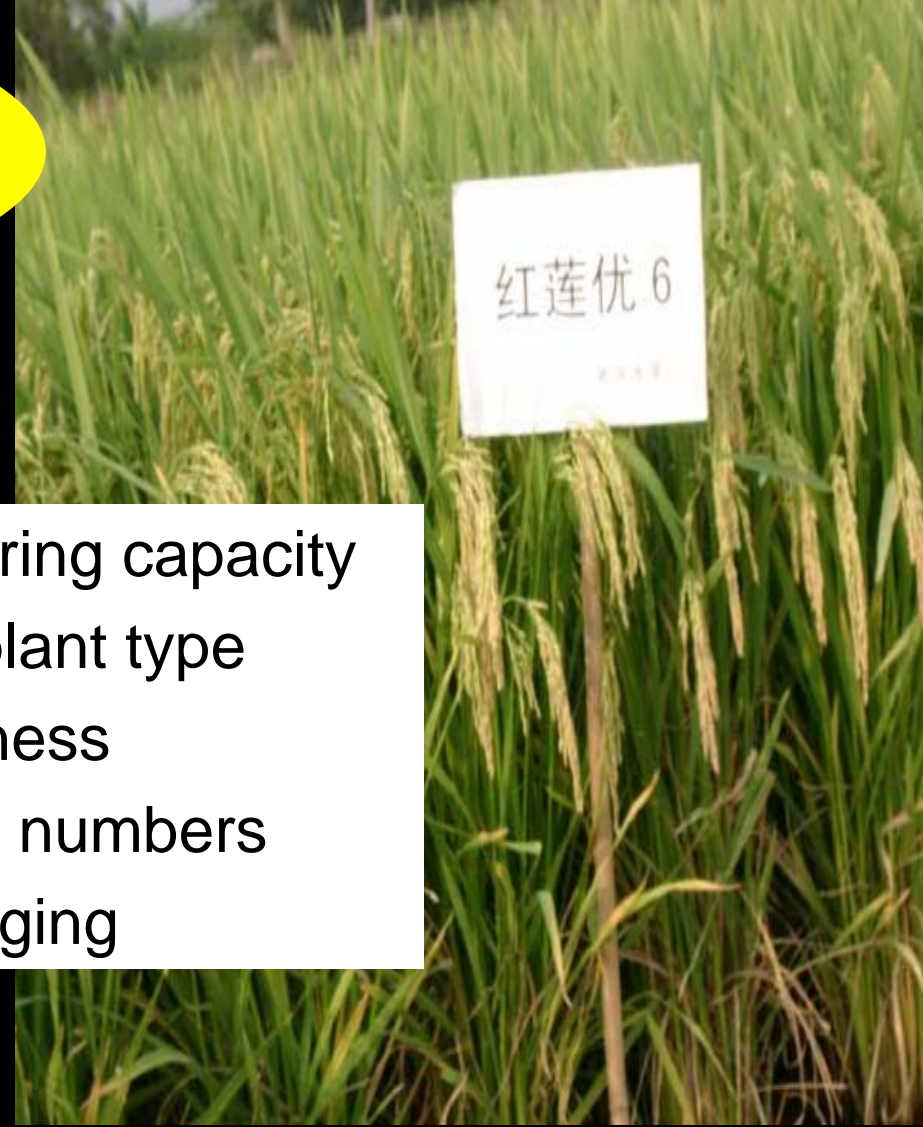
中国水稻产区分布略图



Better performance characters



- Strong tillering capacity
- Compact plant type
- Straw stiffness
- More grain numbers
- Delaying aging



HL hybrid rice well balance the conflict of grain yield and quality

Easy for hybrid seed production

CMS lines have good flowering behavior:

- Longer flowering time
- Higher out-crossing rate
- Synchronous flowering with restorer lines

The yield of hybrid seed production reached to **4.5 tons/hectare**



Novel CMS germplasm development

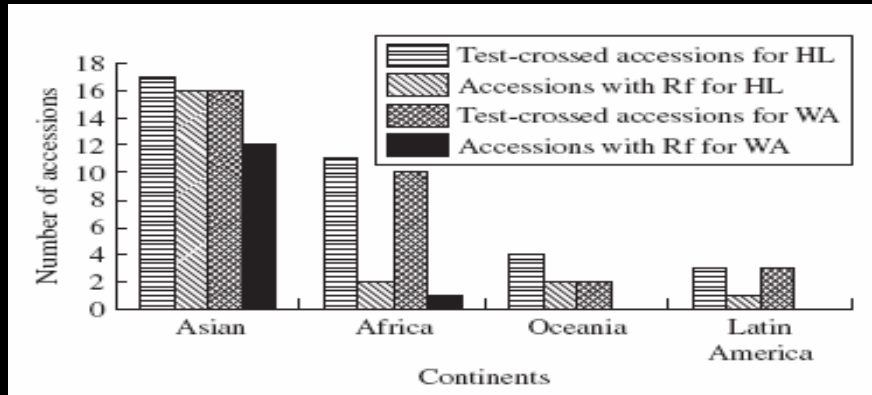
New germplasm development

- ❖ Approaches: Biotechnology combined with conventional breeding methods to improve the agronomic characters of rice variety;
- ❖ Strategy: Screening of novel germplasm and applied to hybrid rice development
- ❖ Resources: Wild rice and native rice varieties

New germplasm development

- ✦ Screen novel Rf gene
- ✦ Develop new molecular markers
- ✦ Screen novel gametophytic cytoplasm
- ✦ Improve resistance

Screening novel *Rf* gene from wild rice species

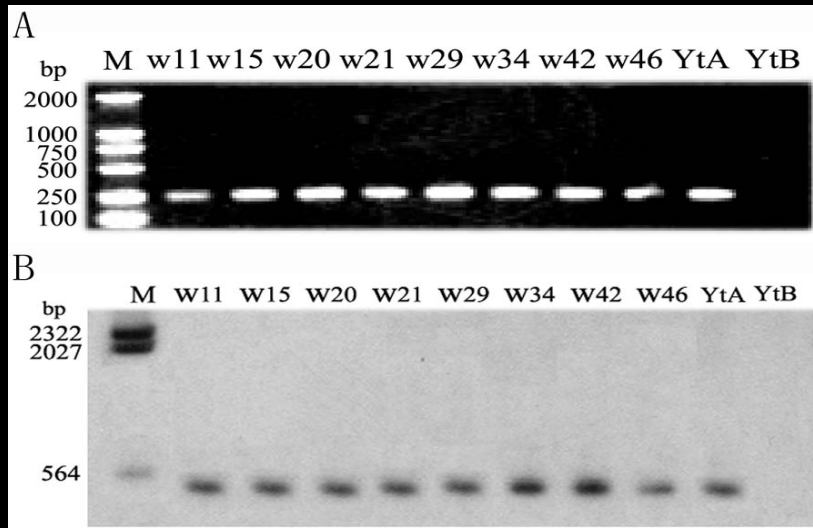


Geographically, *Rf* distributed in Asia, Oceania, Latin American and Africa, but centered mainly in Asia.

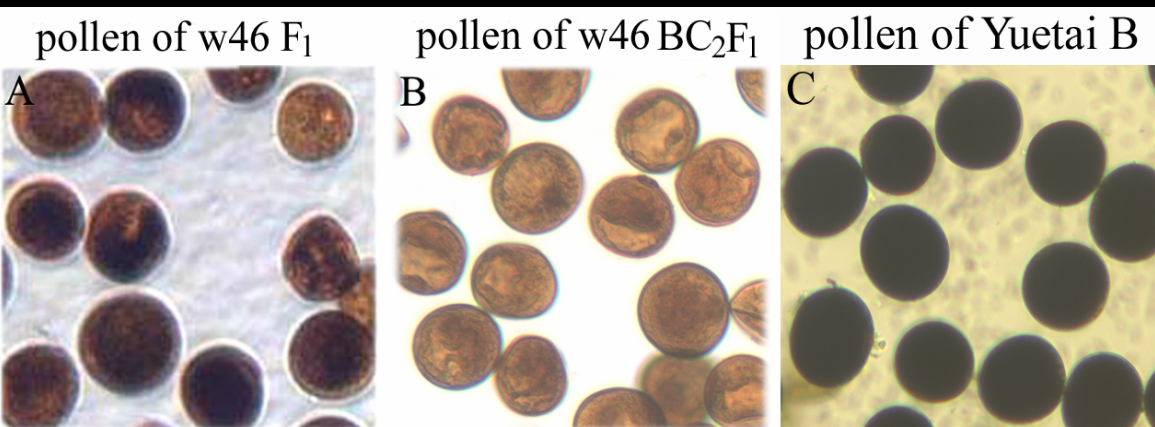
Species	<i>O.barthi</i> <i>i</i>	<i>O.Glaber</i> <i>rima</i>	<i>O.glumaepat</i> <i>ula</i>	<i>O.Meridiona</i> <i>lis</i>	<i>O.nivara</i>	<i>O.rufipo</i> <i>gon</i>
HL-Rf ratio %	16.3	33.3	20	50	100	88.9
WA-Rf ratio %	20	0	0	0	62.5	87.5

Apart from *O. longistaminata*, *Rf* was found in all of the other six wild rice species with AA genome, and aggregated mainly in the two species of *O. rufipogon* and *O. nivara*,

Screening novel gametophytic cytoplasm using orfH79 as molecular marker

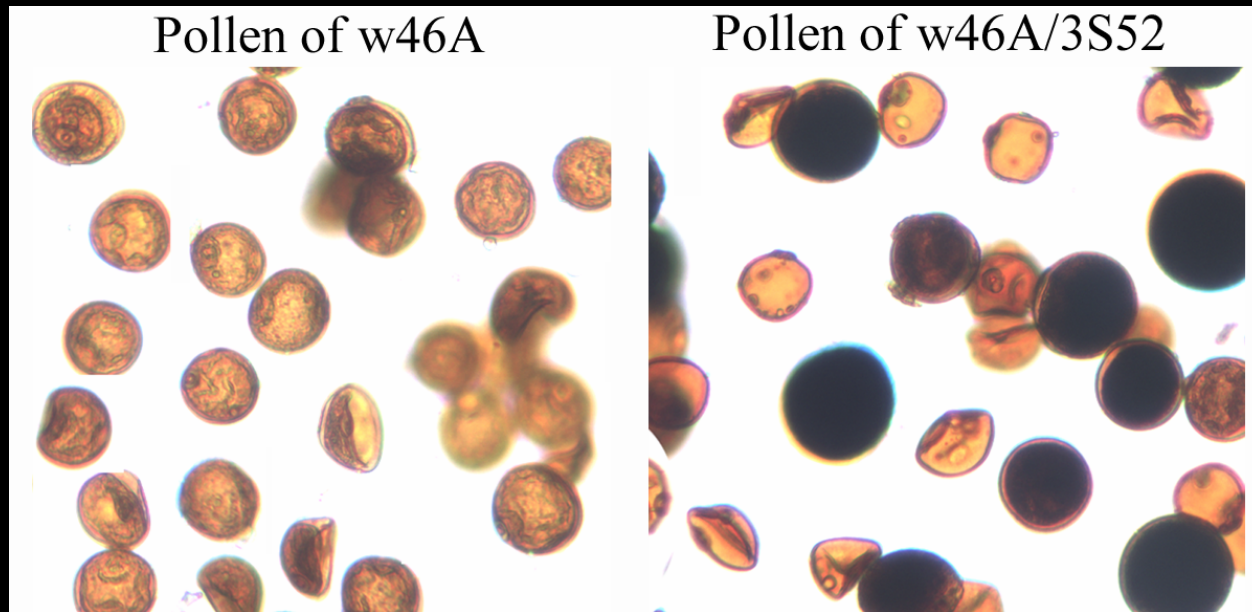


Eight wild rice accessions were obtained by using molecular marker of orfH79 derived from HL-CMS



Pollen phenotype of F₁ and BC₂F₁ from the crosses between wild rice and Yuetai B showed HL CMS character.

Four gametophytic alloplasmic CMS lines were developed via MAS



Four obtained CMS cytoplasms exhibit typical features of gametophytic cytoplasm in CMS line and F1 hybrid

Differences of the Fertility restoring between the four gametophytic alloplasmic CMS lines

Sterile lines	Restorer lines													
	3S52	MBP98	95-102	Huajing74	Gumei2	R644	Yue-xiang-zhan	Zhong413	C418	Feng-xiang-zhan	UP15	M401	MR77	BR24
YtA	^A 30.7±2.1	48.6±3.3	51.3±2.4	60.6±3.1	3.3±0.4	43±3.5	64.3±2.2	10.4±4.7	20.5±3.4	57.3±2.7	0	60.8±2.6	50.1±4.2	0
	^B 41.9±2.7	84.7±4.3	93.8±0.5	88.3±2.7	0	84.6±2.6	85.7±2.9	24.1±1.3	34.3±2.6	81.8±1.8	0	90.5±2.0	87.6±1.7	0
	^C 28.8±2.7	71.5±4.8	73.5±3.9	78.6±3.6	0	59.3±2.8	68.4±3.7	13.7±1.5	29.4±1.8	73.5±2.6	0	68.3±1.8	63.8±2.1	0
w15A	46.7±5.3	23.6±4.1	43.5±0.4	53.3±2.4	12.6±.08	32.5±1.9	5.2±0.1	0	33.5±2.4	42.6±3.4	0	63.4±1.8	43.7±1.2	32.6±1.5
	78.7±5.6	31.3±3.1	3.4±0.3	78.9±2.5	23.5±1.5	57.2±2.5	1.2±0.1	0	57.7±1.7	80.1±2.7	0	86.6±2.9	81.5±1.9	67.6±2.3
	62.1±4.7	23.8±3.8	0	69.4±2.8	26.3±1.1	25.8±1.3	0	0	38.2±1.6	66.4±3.5	0	72.4±2.4	67.4±1.5	54.6±2.4
w20A	44.8±1.8	50.3±2.2	56.9±3.6	15.7±2.1	38.2±1.3	29.6±1.1	3.1±0.1	0	37.4±4.2	56.4±3.8	43.7±1.4	55.8±3.1	53.8±0.8	3.5±0.2
	80.6±1.1	79.4±2.4	83.7±2.7	30.6±1.4	58.2±2.7	61.4±1.9	0	0	61.9±2.9	88.2±2.9	79.5±	87.3±1.6	69.4±2.6	0
	66.1±1.8	60.3±3.1	61.5±3.0	16.4±1.2	53.6±2.2	35.7±1.7	0	0	43.7±1.6	69.3±4.3	50.7±	67.6±2.7	63.7±1.7	0
w34A	60.7±4.8	25.8±2.6	0	44.8±1.5	44.7±2.6	55.3±2.5	21.7±1.2	40.6±2.1	25.7±2.1	47.3±2.1	15.2±1.7	63.7±2.5	4.7±0.1	17.3±1.4
	84.9±4.4	50.8±3.9	0	81.5±2.6	84.2±2.6	83.6±4.2	55.5±	74.7±2.7	54.6±1.4	84.3±3.7	26.1±	79.2±1.8	1.7±0.3	25.6±2.0
	66.4±3.4	41.7±2.4	0	63.9±1.5	56.8±3.1	50.7±2.3	18.4±	57.3±2.6	23.8±2.1	63.6±2.6	12.7±	64.8±2.2	0	18.5±1.5
w46A	54.2±3.7	18.4±2.2	37.7±2.0	48.1±0.6	8.2±0.2	57.2±2.1	35.7±1.7	7.2±1.3	37.2±2.9	35.8±1.3	0	45.8±1.9	68.3±4.9	13.8±1.6
	87.5±5.2	33.1±1.9	87.7±4.8	78.3±2.3	11.5±1.0	80.6±2.7	61.4±2.5	6.3±0.6	57.2±2.9	55.7±2.4	0	81.2±2.5	85.9±2.4	21.6±1.9
	71.4±4.5	34.4±3.3	70.8±4.5	54.8±1.8	2.1±0.3	53.7±3.4	24.9±1.7	3.1±0.3	20.4±2.7	31.6±2.6	0	43.8±2.8	57.7±1.7	12.7±1.1

Crossing results indicated that four gametophytic alloplasmic CMS lines showed different fertility restoring patterns

Improvement of resistance of restorer and HL-CMS lines by introducing Bph14, Bph15 and Pi-9 genes



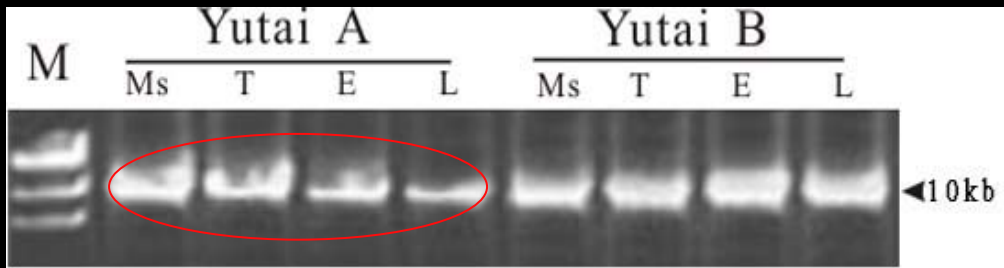
We are developing several HL-CMS and fertility restorers lines that anti-brown plant hopper (Bph14 and 15) and rice blast gene pi-9 by using MAS



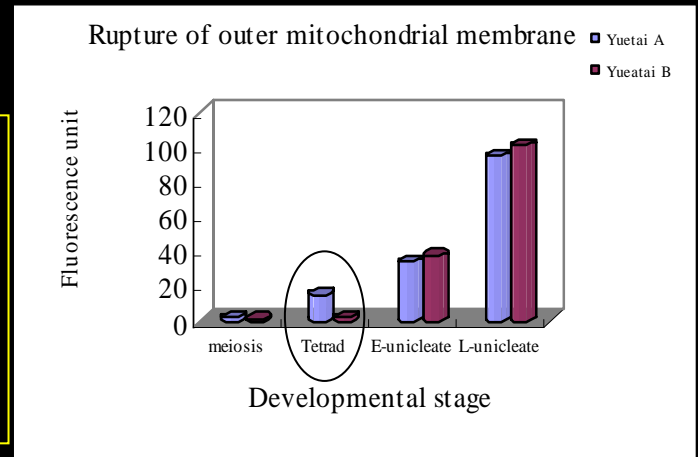
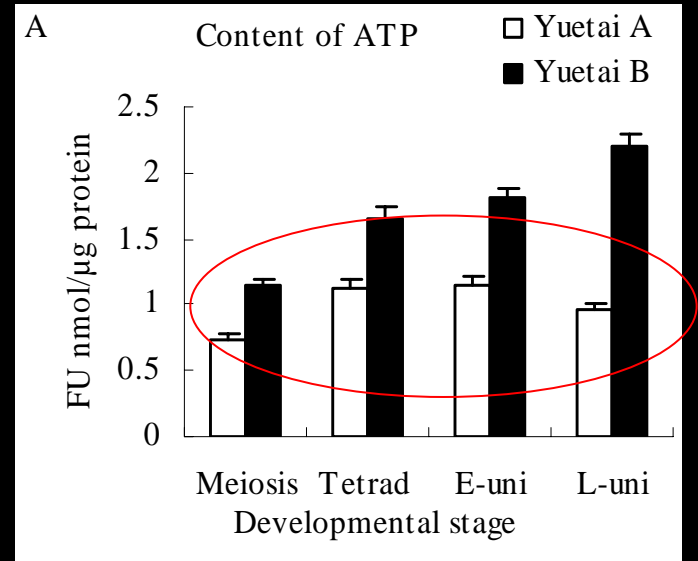
Mechanism of HL-CMS

Dysfunction of mitochondria in the pollen development of HL-CMS

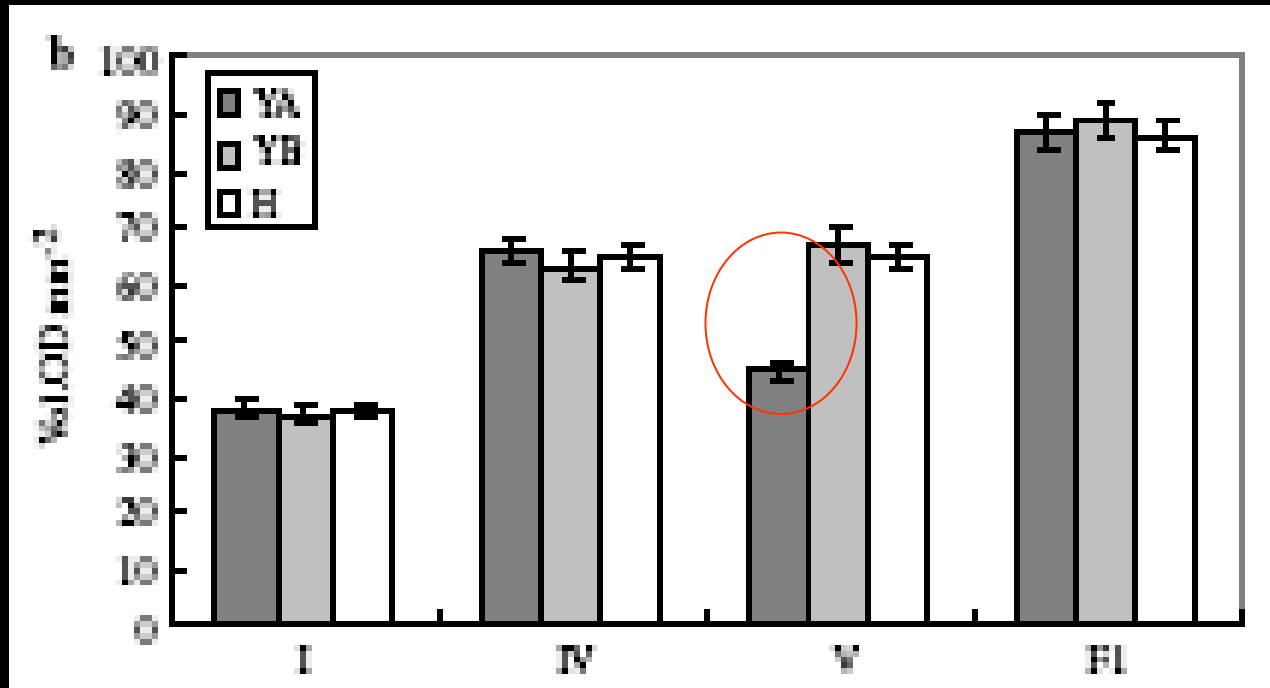
PCR detect the breakage in the mt DNA



Increase of abnormal ROS impaired the DNA, contents of ATP and membrane potential in the mitochondria of microspores of HL-CMS line



Reduction of complex V activity in the male sterile line



In-gel enzyme activities of mitochondria respiratory complexes showed Reduction of complex V activity in the male sterile line

Mitochondria impairment is getting severe during microspore development

YtB

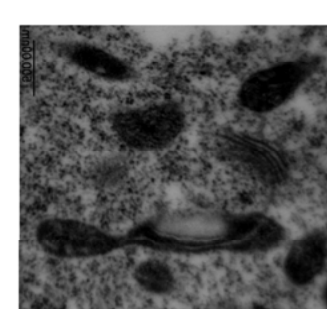
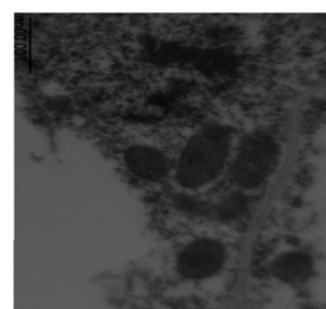
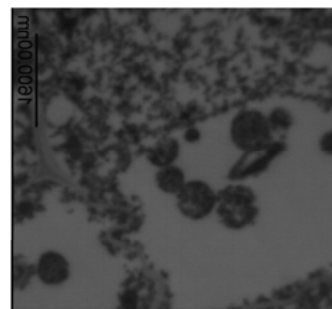
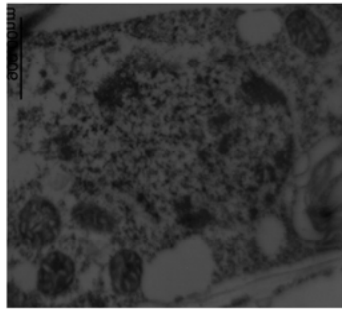
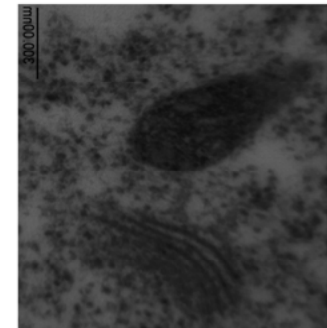
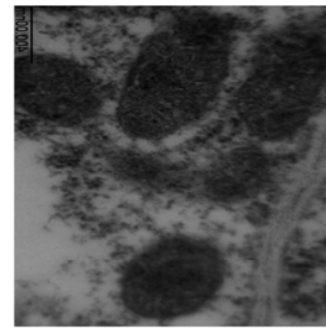
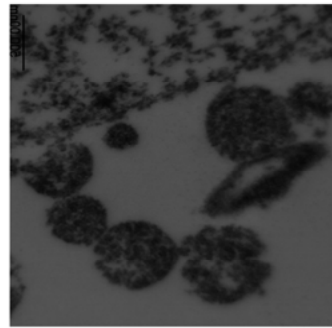
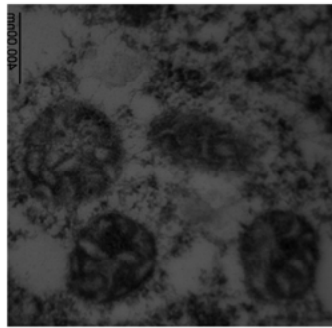
YtA

Tetrad

uninucleate

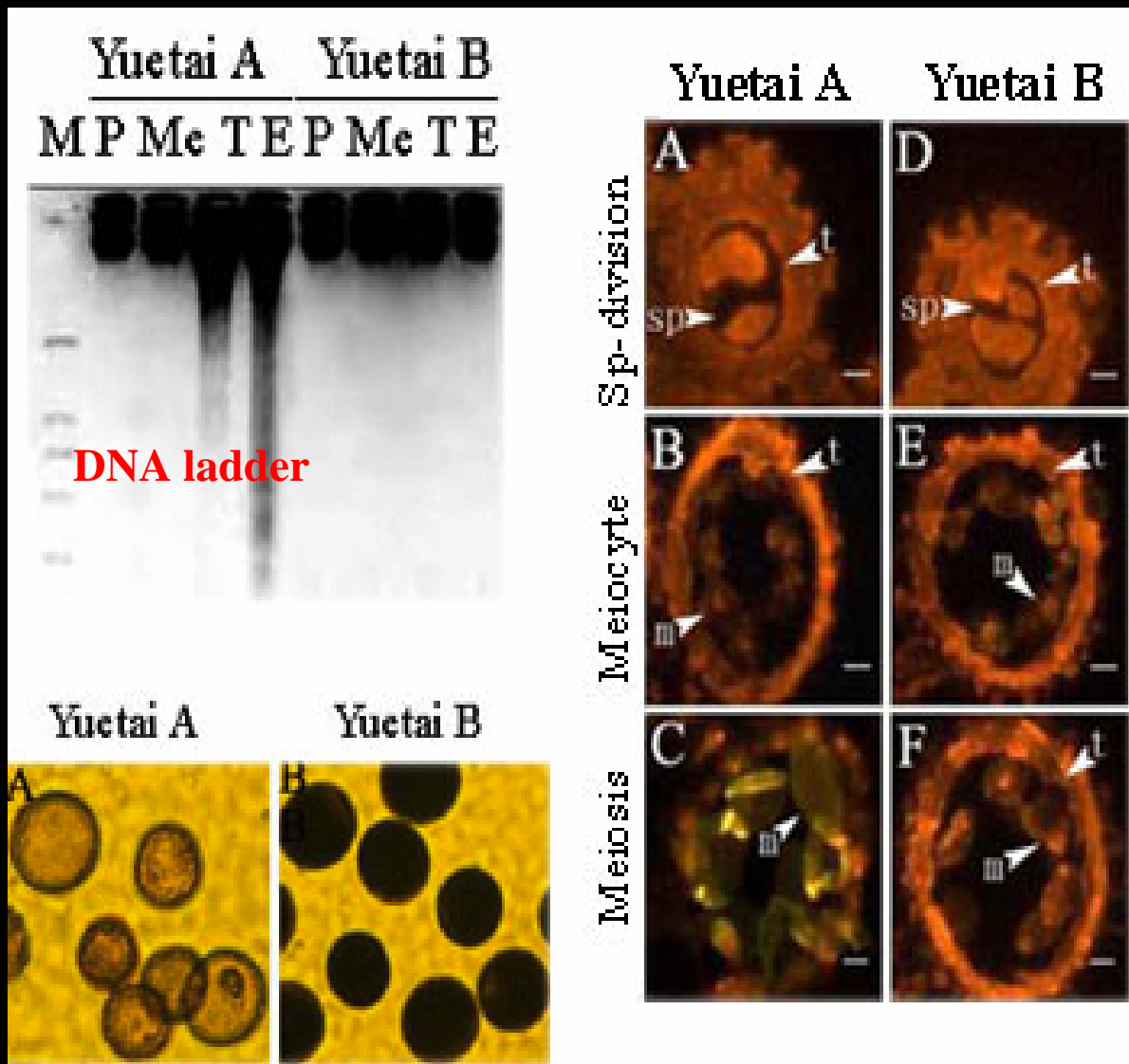
Tetrad

Pollen
mother cell



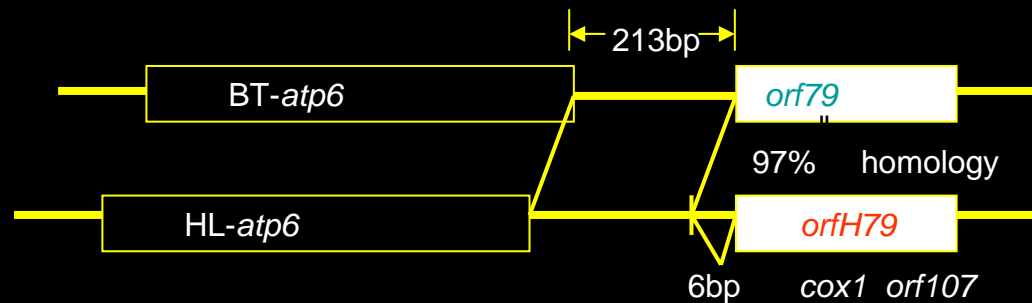
Microstructure observation revealed that the mitochondrial membrane is getting blur during the microgenesis in HL-CMS line

Microspores exhibit abnormal PCD phenomenon in HL-CMS lines



TUNNEL analysis and DNA ladder detection revealed the nuclear DNA breakage in meiosis stage, suggesting a premature PCD occurrence.

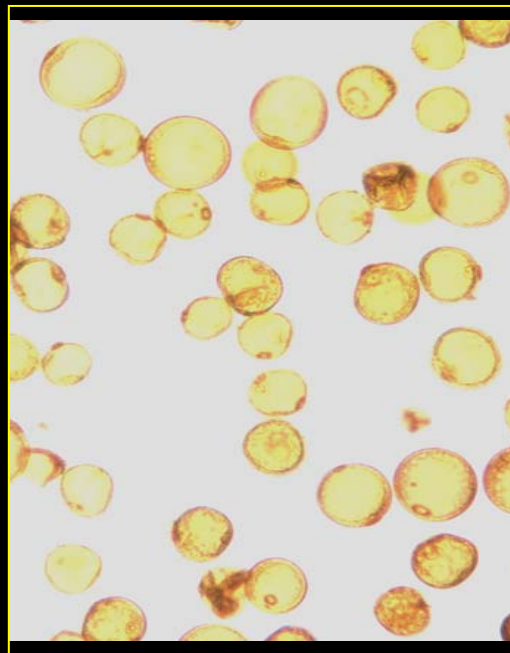
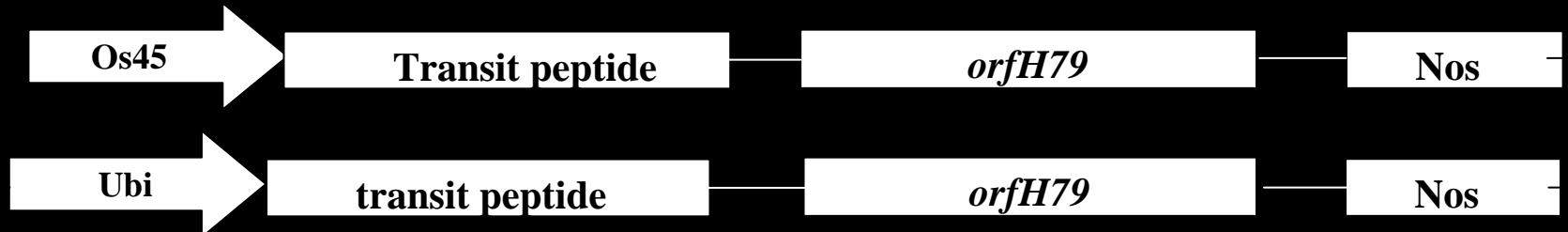
Structure of orfH79 and orf79 have difference in HL and Boro-CMS



Five nucleotide mutations of *orfH79* resulted
in five amino acids alteration over *orf79*

Transgenic rice of *orfH79* Mimic the male sterile phenotype

orfH79 recombinant vector



PCR identification showed that the ratio of sterile and fertile plants is about 1:1 in T1 generation

Genetic analysis of Fertility restorer (*Rf*) for HL-CMS

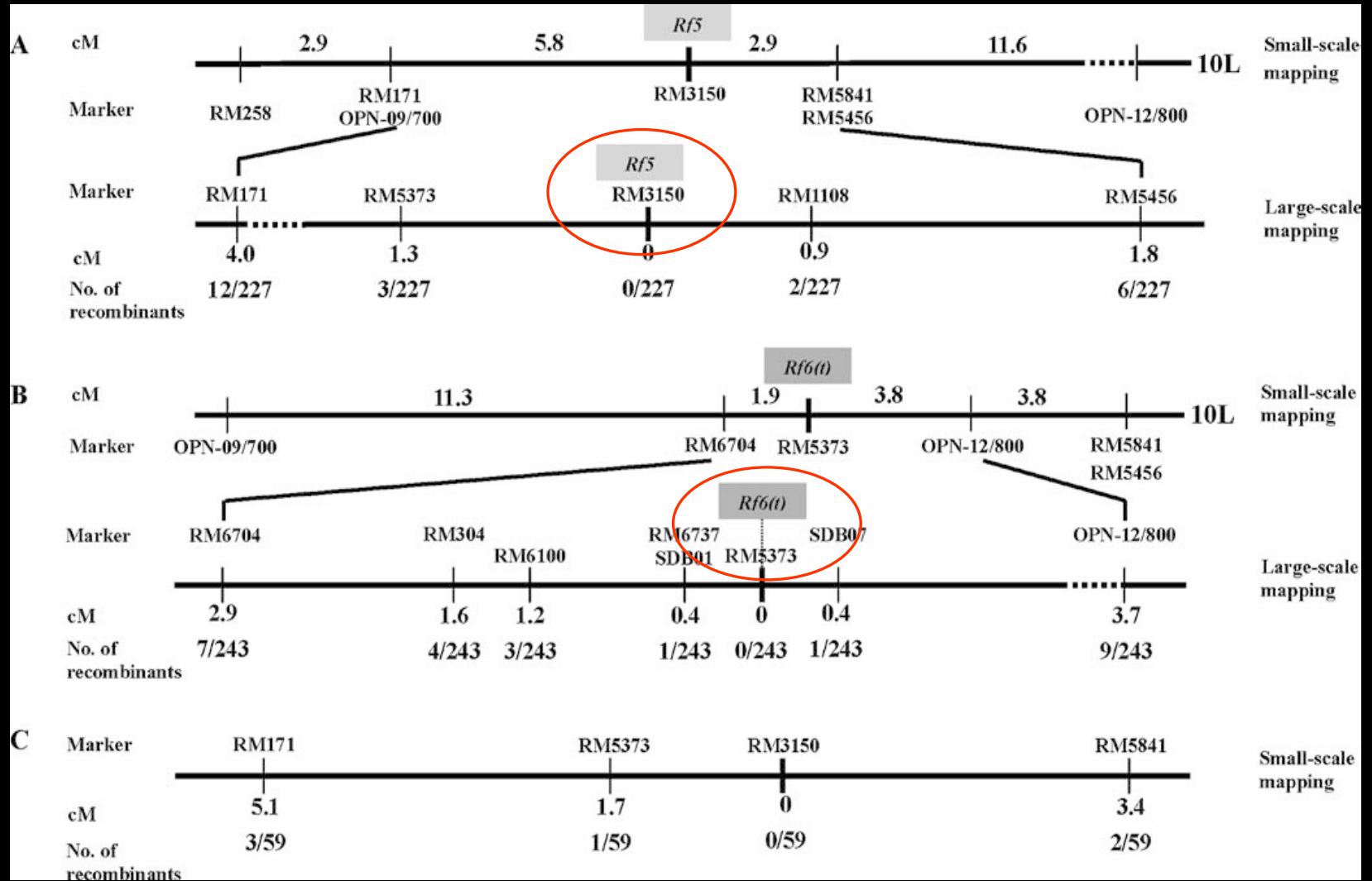
Segregation of fertile plants and sterile plants in BCF₁

Crosses	plants scored	fertile plants	sterile plants	χ^2 value for 1:1 segregation	<i>P</i>	Recombination rate (%)
CG-41A//CG-41B/MY23	863	451	412	0.8812	0.250-0.500	-
YTA //YTB / MY23	250	134	116	0.6480	0.250-0.500	-
YTA//YTB/9311	560	298	262	1.1571	0.250-0.500	-
YTA//MY23/9311	70	67	3	58.5143 (16.0191) ^a	< 0.005	8.57

^a The value for 3:1 segregation is shown in *parentheses*

Genetic analysis showed fertility of HL-CMS is controlled by *Rf5* or *Rf6* genes

Fine mapping of *Rf5* and *Rf6(t)* for HL-CMS



Perspectives

In the 21th century, Hybrid rice is playing important role for increase of grain yield. The tendency of hybrid rice development will focus on higher grain yield, better grain quality, more resistance and higher efficiency of nutrient utilization. Honglian hybrid rice fits to those trends and shows high potential.

Honglian hybrid rice has bright future for increasing food production and benefit the world

Acknowledgements

- Dr. Kim for kindly providing Miyang23 BAC clones
- Wuhan University:
 - Supervisor: Yingguo Zhu
 - Daichang Yang
 - Jun Hu/Wenchao Huang
 - Shaoqing Li/Yanping Tan
 - Xiaojue Peng/Chaofeng Hu
 - Renshan Zhu

Thanks for attention !