

Utilization of wild species in hybrid rice breeding

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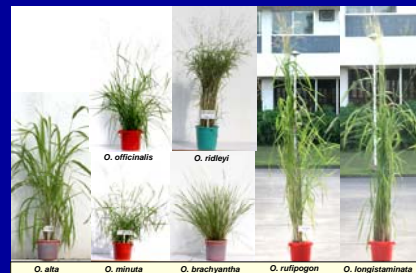
Major objectives in hybrid rice breeding

1. Increased heterosis/grain yield (10-20% over the best inbred)
2. Stable male sterility systems (CMS, TGMS, PGMS)
3. Multiple resistance to diseases, insects and abiotic (environmental) stresses
4. Improved grain quality
5. Increased hybrid seed yield- (good restoration and out crossing)-reduced seed cost
6. Pre-breeding to develop heterotic genepools/ gene blocks/WC genes

Outline

1. Broadening gene pool of parental lines of hybrid rice
2. CMS diversification and enhancing out crossing
3. Introgression of favorable wild species alleles: enhancing yield of parental lines
4. Identification of heterotic gene blocks/ chromosome regions
5. Identification of heterotic combinations involving crosses with alien introgression lines
6. Enhancing genetic diversity using distant hybridization- pollen tube pathway (?)
7. Exploring possibility to develop one line system (**apomixis**)

Wide hybridization



Useful Traits

- Insect resistance
- Disease resistance
- Tolerance to abiotic stresses
- QTLs for yield

Genes for resistance to BPH (*Bph10*, *Bph18*, *Bph(t)*), BB (*Xa21+* unknown), blast (*Pi9*, *Pi40*), tungro, grassy stunt and tolerance to acid sulfate, iron toxicity and CMS have been transferred from wild species into rice at IRR

Strategies for gene transfer from wild species

- Search for useful genetic variability
- Production of hybrids/alien introgression lines
- Evaluation of introgression lines for target traits under lab, screenhouse and field conditions
- Characterization of introgression using molecular markers
- Tagging/pyramiding of introgressed genes /QTLs for MAS
- Location of introgressed segments through GISH/FISH
- Fine mapping/isolation of genes/QTLs using BAC libraries
- Functional genomics: allele mining and gene expression

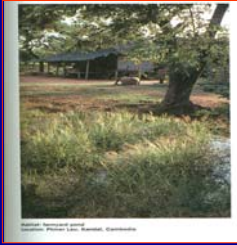
Wide-cross progenies developed at IRR

Cross Combination	F1	Mapping population(s)	Advanced Introgression lines	Cytogenetic stocks*
<i>O.sativa</i> x <i>O. rufipogon</i> (AA)	+	++	+++	-
<i>O.sativa</i> x <i>O. glaberrima</i> (AA)	+	+++	++++	-
<i>O.sativa</i> x <i>O. longistaminata</i> (AA)	+	+	+	+
<i>O.sativa</i> x <i>O. officinalis</i> (CC)	+	+	+	+
<i>O.sativa</i> x <i>O. minuta</i> (BBCC)	+	+	+	+
<i>O.sativa</i> x <i>O. latifolia</i> (CCDD)	+	+	+	+
<i>O.sativa</i> x <i>O. alta</i> (CCDD)	+	+	-	+
<i>O.sativa</i> x <i>O. australiensis</i> (EE)	+	+	+	+
<i>O.sativa</i> x <i>O. brachyantha</i> (FF)	+	+	+	+
<i>O.sativa</i> x <i>O. granulata</i> (GG)	+	+	+	+
<i>O.sativa</i> x <i>O. ridleyi</i> (HHJJ)	+	+	+	+
<i>O.sativa</i> x <i>O. coartata</i> (HHKK)	+	+	-	-

Hybrids and progenies have been produced across crossability barriers through embryo rescue between rice and all the 10 genomic types of *Oryza*.

* MAALS

Grassy stunt resistance



Source: Vaughan, 1994

- Of 6700 lines, only one accession (*O. nivara*) was found to be resistant
- Resistance bred into many IR cultivars

Look for resistance in wild species for newly emerging diseases – False smut !

Broad spectrum resistance of introgression lines derived from *O. sativa* x *O. australiensis* to 13 *P. grisea* isolates of Korea

Parent/introgression line	Korean isolates												
	1	2	3	4	5	6	7	8	9	10	11	12	13
IR31917-45-3-2 (parent)	S	S	S	S	S	S	S	S	S	S	S	S	S
IR65482-4-136-2-2 (introgression line)	R	R	R	S	R	R	R	R	R	R	R	R	R
IR65482-17-511-5 (introgression line)	R	R	S	R	R	R	S	S	S	R	R	R	R

Eung-Gi-Jeong et al., 2004

O. rufipogon (WBB1) resistant to 9 Philippines races of BB (Zhang, et al, 2003)
O. rufipogon (106407) resistant to 10 Philippines races of BB (IRRI, unpublished)
O. longistaminata (110404) resistant to 9 Philippines races of BB (IRRI, unpublished)

Introgression of agronomically important genes from wild species into rice at IRRI

Trait transferred	Donor <i>Oryza</i> Species	
	Wild species	Genome(s)
Grassy stunt resistance	1	AA
Bacterial blight resistance	6	AA, BBCC, CC, CCDD, EE, FF
Blast resistance	2	BBCC, EE
Brown plant hopper resistance	4	CC, BBCC, CCDD, EE
Whitebacked plant hopper resistance	1	CC
Tungro tolerance	1	AA
Tolerance to acid sulfate conditions	1	AA
Cytoplasmic male sterility	2	AA

IRRI breeding lines released as varieties through wide hybridization



BPH resistant (Vietnam) *O. officinalis*



Tungro disease (Philippines) *O. rufipogon*



High yielding (Philippines) *O. longistaminata*



A popular variety in rice grown on 100,000 ha in Mekong Delta (Vietnam) *O. rufipogon*



Japonica rice x *O. glaberrima* (YAAS, China)



A high yielding hybrid with *Xa 21* gene released by CNRRI, China

Introgressed genes from wild species tagged with molecular markers

Trait	Gene	Species
BPH resistance	<i>Bph 10, Bph18</i>	<i>O. australiensis</i>
	<i>bph 11(t), 12(t)</i>	<i>O. officinalis</i>
	<i>BPH 13t (?)</i>	<i>O. minuta</i>
BB resistance	<i>Xa21</i>	<i>O. longistaminata</i>
	<i>Xa-23</i>	<i>O. rufipogon</i>
	<i>Xa24(t)</i>	<i>O. minuta</i>
Blast resistance	<i>Pi-9(t)</i>	<i>O. minuta</i>
	<i>Pi-40</i>	<i>O. australiensis</i>
Tungro resistance	RTSV (?)	<i>O. rufipogon</i>
Aluminum toxicity tolerance	QTL	<i>O. rufipogon</i>

Pyramiding of genes for BB resistance using MAS

Gene(s)	Bacterial blight races					
	1	2	3	4	5	6
Parent (susceptible)	S	S	S	S	S	S
Xa-4	R	S	S	S	R	S
Xa-13	S	S	S	S	S	R
Xa-21	R	R	R	R	R	R
Pyramided lines						
IRBB56 (Xa-4+xa-5+xa-13)	R+	R	R	R+	R+	R
IRBB59 (xa-5+xa-13+Xa-21)	R+	R+	R+	R+	R+	R
IRBB60 (Xa-4+xa-5+xa-13+Xa-21)	R+	R+	R+	R+	R+	R+

MAS products released as rice varieties - Xa21 gene from wild species pyramided with other BB resistance genes

Variety	Genes for BB resistance	Country
NSIC142 (PR31563-AR32-19-3-3)	Xa4/Xa21	Philippines
NSIC154 (PR31561-AR32-11-83-1-4-1)	Xa4/Xa21	Philippines
Improved Pusa Basmati	Xa21+ others	India
Improved Samba Mahsuri	Xa21 + others	India
3 commercial hybrids	Xa4/Xa21	China

Transgenic (GM) rice



Field testing of transgenic IR72 with Xa21 gene (source: *O. longistaminata*) for bacterial blight resistance, IIRRI Experimental Station, Los Baños, Laguna, Philippines



Field evaluation of transgenic rice in China with Xa21 gene showing resistance to bacterial blight (Tu et al. 2000 Theor. Appl. Genet. 101:15-20)

Hybrid rice breeding



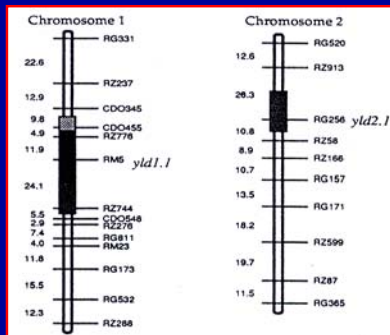
- A male sterile wild rice plant with abortive pollen found in 1970 in Hainan Island, China and designated as wild abortive (WA)
- used extensively world wide in hybrid rice breeding

Source: Lin Shih-Cheng and Yuan Longping (1970)

Cytoplasmic diversification

CMS lines	Recurrent rice parent	Donor wild species	Remarks
IR66707A	IR64	<i>O. perennis</i> ^a (Acc. 104823)	No good restorer (s)
IR69700	IR64	<i>O. glumaepatula</i> ^a (Acc. 100969)	No good restorer (s)
RPMS1	IR66, RMS2B	<i>O. rufipogon</i> ^b	No good restorer (s)
RPMS3	IR66*	<i>O. nivara</i> ^b	No good restorer (s)

^a IIRRI; ^b DRR, Hyderabad, India



Yield enhancing loci (*yld 1* and *yld 2*) identified in wild species (Nature 1998, Genetics 1998, Theor. Appl. Genet. 2001, 2003,2003; Next steps: 1. transfer into high yielding cultivars through MAS; 2. Fine mapping and isolation of QTLs

Chromosome segmental substitution lines - CSSL



Takai et. al (2007)

SL514 x SL544 → look for heterosis?

Wild species of *Oryza* screened for apospory (multiple embryo sac development) and diplospory (callose fluorescence) types of apomixis


Species	Genome	Accessions (no.) analyzed for:	
		Apospory	Diplospory
Diploid			
<i>O. sativa</i>	AA	3	3
<i>O. nivara</i> , <i>O. longistaminata</i> , <i>O. barthii</i> , <i>O. glumaepatula</i>	AA	12	6
<i>O. punctata</i>	BB	2	2
<i>O. officinalis</i>	CC	5	3
<i>O. australiensis</i>	EE	6	3
<i>O. brachyantha</i>	FF	3	3
<i>O. granulata</i>	GG	4	2
Total		35	22
Tetraploids			
<i>O. punctata</i> , <i>O. minuta</i> , <i>O. malampuzhaensis</i>	BBCC	51	45
<i>O. alta</i> , <i>O. latifolia</i> , <i>O. grandiglumis</i>	CCDD	47	33
<i>O. ridleyi</i> , <i>O. longiglumis</i>	HHJJ	10	8
		108	86

No evidence of apomixis Khurshid et al (1998)

Enhancing outcrossing using *O. longistaminata* as donor

Species	Stigma (mm)	Anthers (mm)	No. of pollen grains/anther
<i>O. sativa</i>	0.4-1.6	0.9-2.8	700-2500
<i>O. longistaminata</i>	2.5	5.5	7000

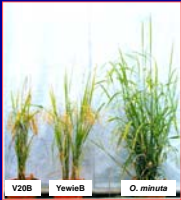
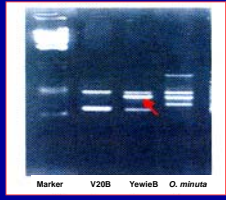
Tallebois & Guimaraes (1998)



O. sativa x *O. longistaminata* (F1)

Distant hybridization through pollen tube pathway

Recipient	Genomic DNA	Reference (s)
Rice	Sorghum	Zhou et al 1986
V20B	<i>O. minuta</i>	Zhao Bingran & Yuan
R207	<i>Echinochloa crusgalli</i>	Longping (2005)

Originally proposed by Pena, et al., 1987, Nature 325:274-276

Construction of BAC libraries from wild species

Species	Genome	Clones	insert size (Kb)
<i>O. nivara</i>	AA	55,296	161
<i>O. rufipogon</i>	AA	64,512	134
<i>O. glaberrima</i>	AA	55,296	130
<i>O. punctata</i>	BB	36,864	142
<i>O. officinalis</i>	CC	92,160	141
<i>O. minuta</i>	BBCC	129,024	125
<i>O. alta</i>	CCDD	92,160	133
<i>O. australiensis</i>	EE	92,160	153
<i>O. brachyantha</i>	FF	36,864	131
<i>O. granulata</i>	GG	73,728	134
<i>O. ridleyi</i>	HHJJ	129,024	127
<i>O. coarctata</i>	HHKK	147,456	123

• Valuable genetic resource for fine mapping and cloning of introgressed alien genes /QTLs Ammiraju et. al 2006
Genome Research 16:140-147

- Wild species utilization in hybrid rice breeding: future priorities**
1. Broaden gene pool of parental lines for tolerance to stresses and pyramid genes/QTLs using MAS
 2. Enhancing outcrossing using *O. longistaminata* for increased hybrid seed yield
 3. Identification and introgression of yield enhancing loci 'favorable wild species alleles' into high yielding parental lines/restorers
 4. Identification and introgression of chromosomal segments/regions responsible for heterosis (look for intercrosses of CSSLs?)
 5. Identification of heterotic combinations involving crosses with alien introgression lines carrying wild species segments
 6. Explore possibility to develop one line system for hybrid rice breeding
 7. Explore possibility to introduce genes from distant crosses using genomic DNA from wild species to enhance diversity-possibly heterosis

Thank you for your kind attention

謝謝您親切的關注
(谢谢您亲切的关注)