6th International Hybrid Rice Symposium,
Hyderabad 10-12 Sept, 2012

Introgressions from *Oryza rufipogon* into the restorer line KMR3 increase hybrid rice yield

N Sarla

www.drricar.org

Directorate of Rice Research

Outline

- Background - why wild?
- Mapping yield enhancing QTLs from *O. rufipogon* - *yld 2.1* sub QTL3 helps increase yield
- Elite introgression lines for several traits
- Elite introgression lines as restorers increase yield of hybrids
- Conclusion

Genetic options to increase yield

- Crosses between varieties
- Hybrids (two-line hybrids, three line hybrids)
- Intraspecific crosses (*indica-japonica-aus-aromatic*)
- Crosses between the two cultivated species (NERICA)
- Crosses between cultivated and wild species

increasing
genetic
diversity

…..new genetic variation accelerates breeding for yield

Wild species help increase yield in cultivated species phenotype not always a good predictor of genotype

Tomato - Wild species with *small fruit* can contribute genes for increase in fruit weight QTLs mapped from several wild species

Rice - Wild species with *low yield* can contribute genes for increase in yield (18%)
Xiao etal 1996 Nature 384, 223

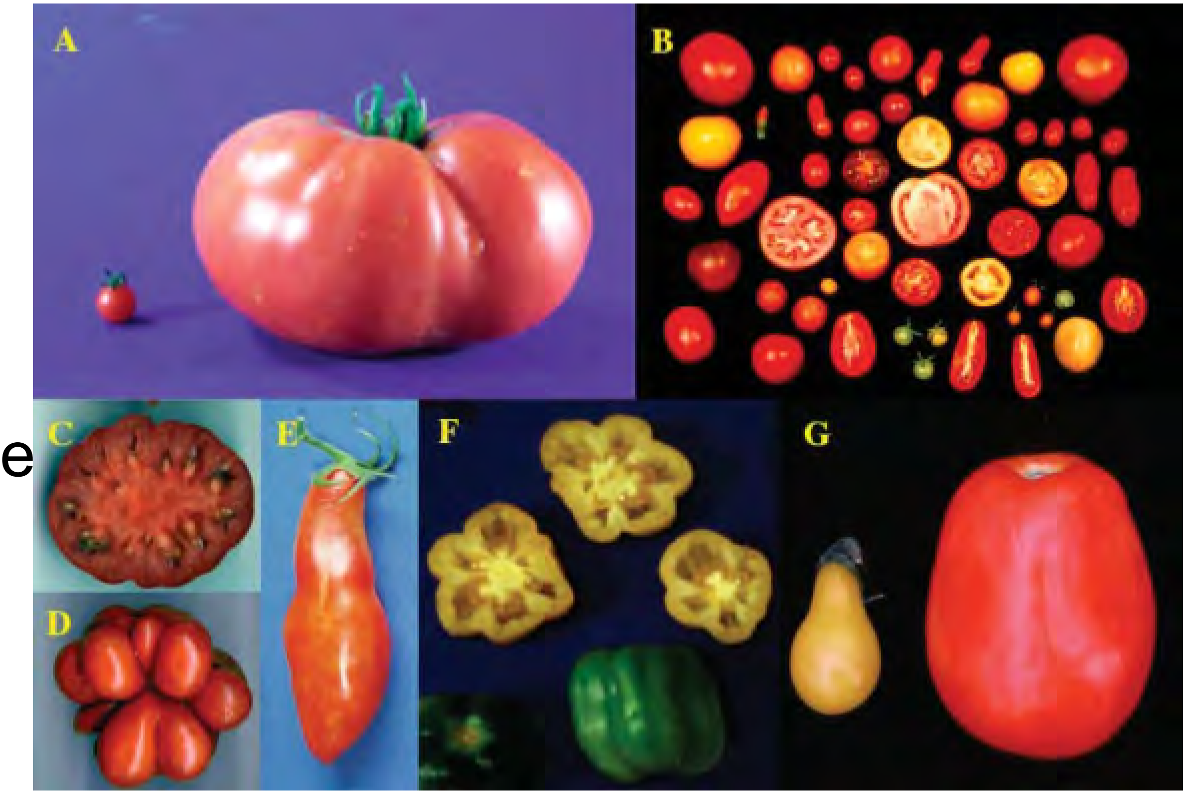
Many wild species help increase yield in tomato

Figure 3. Leaf (A) and fruit (B) phenotypes of the *S. lycopersicum* complex. (I) *S. chmielewskii*, (II) *S. habrochaites*, (III) *S. lycopersicum*, (IV) *S. pimpinellifolium*, (V) *S. neorickii*, and (VI) *S. pennellii*.

Increase in yield, redness, total soluble solids

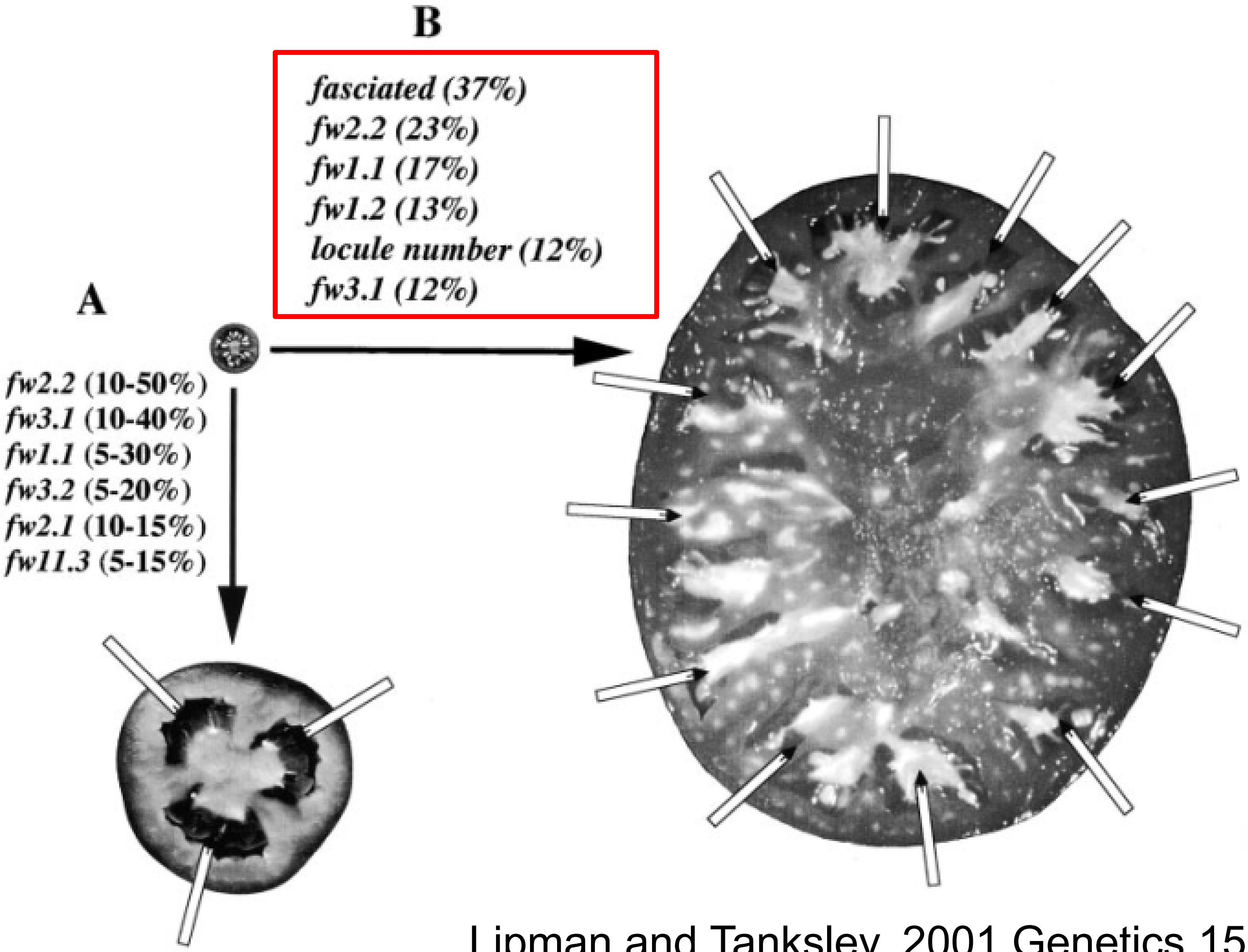
Wild to cultivated

What are the underlying genetic, molecular, and developmental changes that permitted wild progenitors to produce the large, highly variable fruit size shape of tomato ?



Tanksley, SD. 2004. The Plant Cell,16

Six QTLs could explain the genetic variation in fruit size



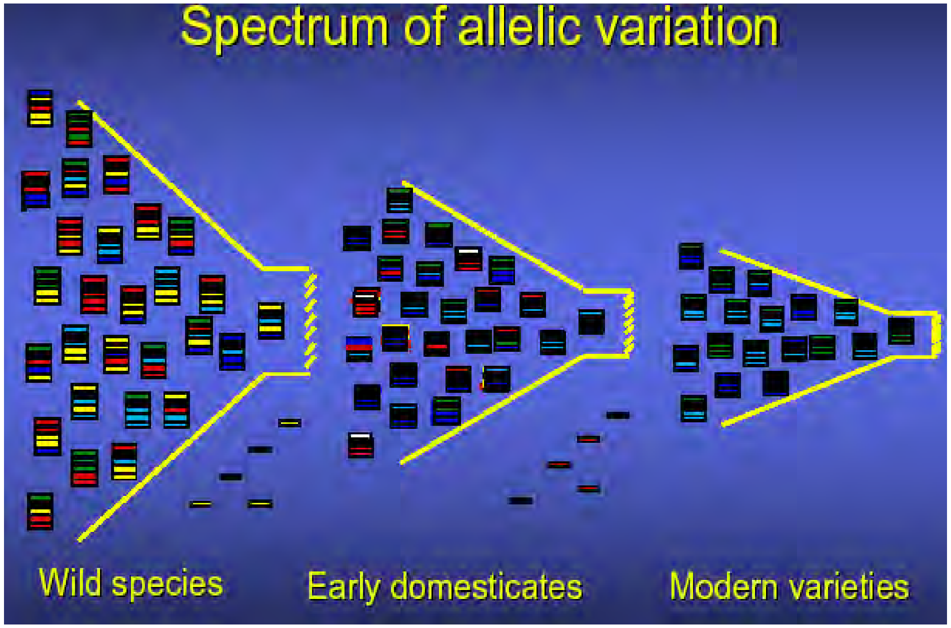
fasciated (37%)
fw2.2 (23%)
fw1.1 (17%)
fw1.2 (13%)
locule number (12%)
fw3.1 (12%)

fw2.2 (10-50%)
fw3.1 (10-40%)
fw1.1 (5-30%)
fw3.2 (5-20%)
fw2.1 (10-15%)
fw11.3 (5-15%)

Lipman and Tanksley. 2001.Genetics 158: 413–422

Natural allelic variation in wild species

Wild species - a good source to increase yield
eg in sugarcane, oats, tomato



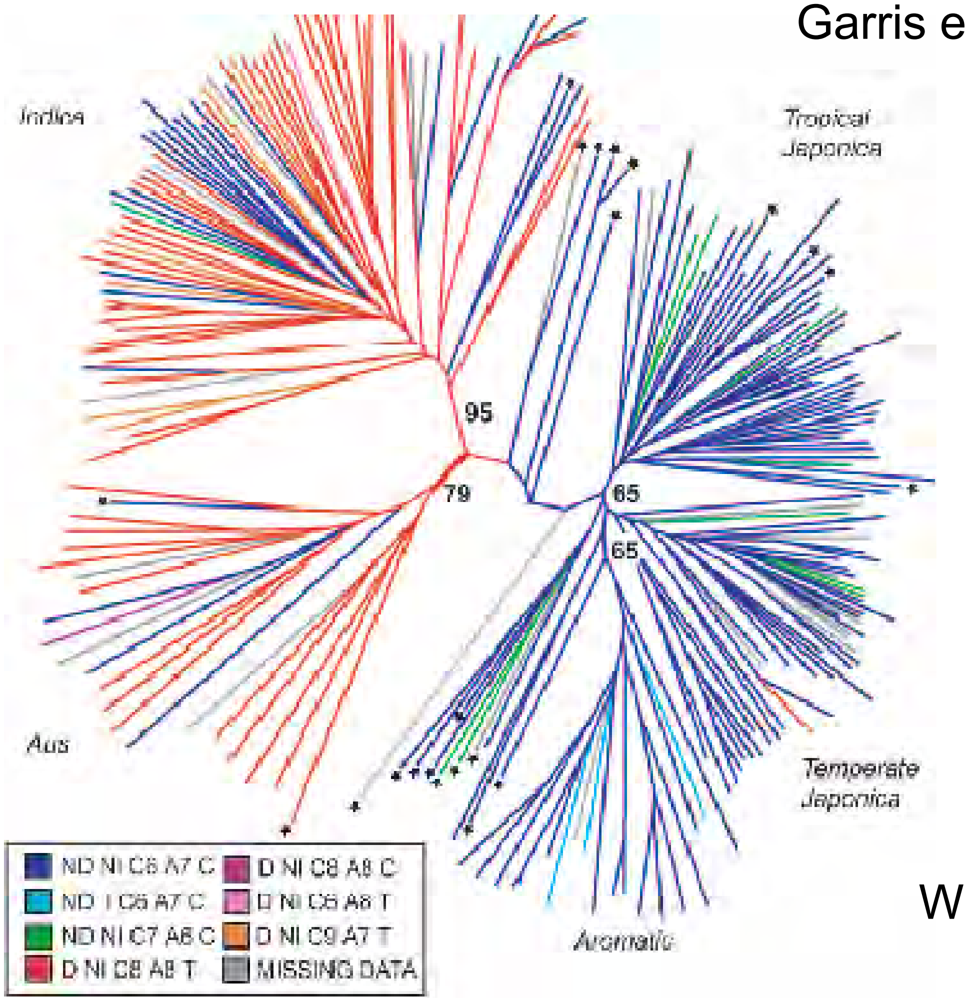
Tanksley and McCouch 1997 Science 277, 1063

- *existing and **created de - novo***
- *35% genome is transposons and retrotransposons*
- *genome is in a flux*

….it is estimated that 80% allelic diversity is still untapped

Rice population structure shows 5 sub groups

Garris et al 2005. Genetics **169:** 1631

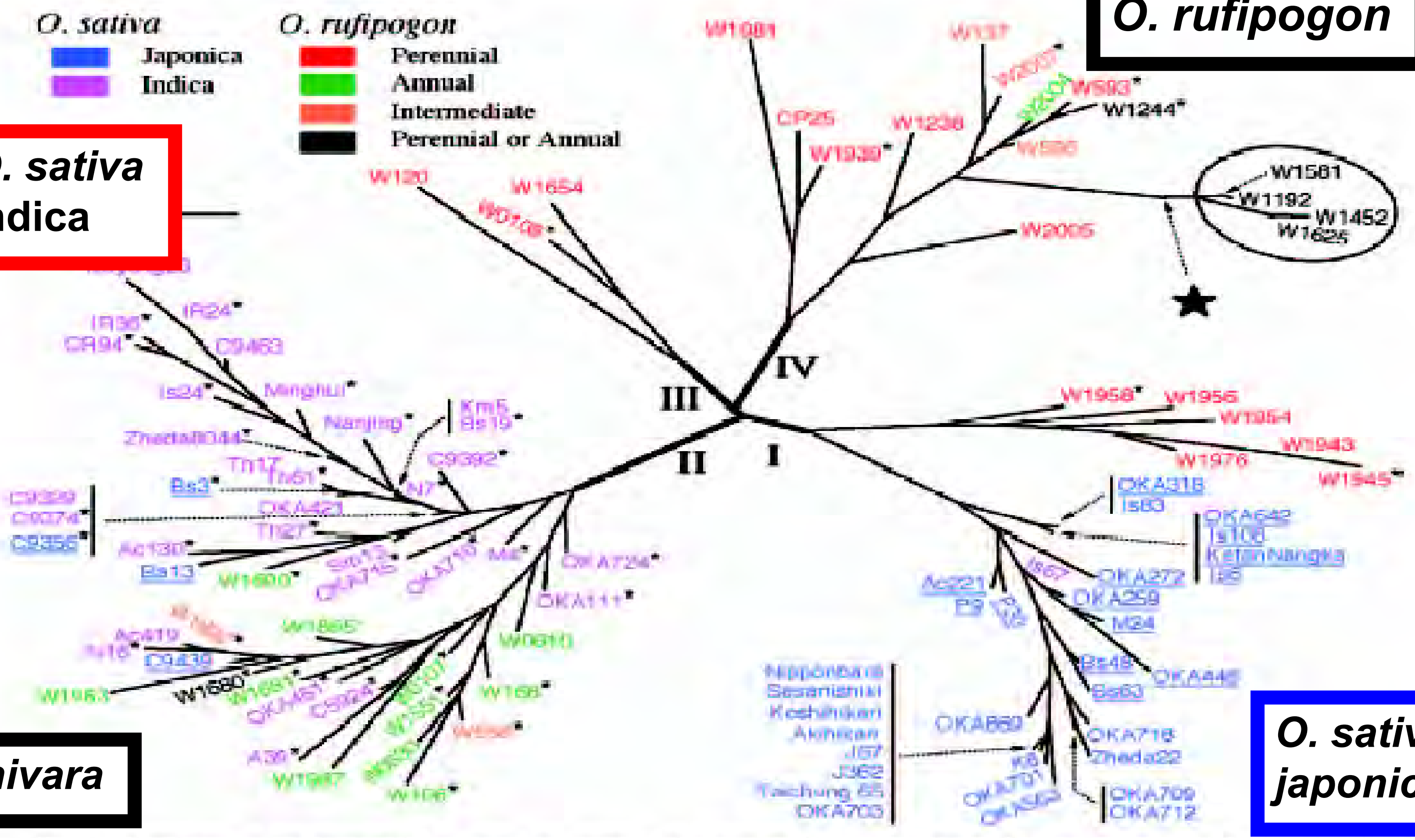


indica x indica
japonica x japonica
indica x japonica

aus
aromatic
temperate japonica

Wild species- are there subpopulations ?

Cheng et al 2003. *Mol Biol Evol (2003) 20, 67-75*



O. rufipogon

O. sativa indica

O. nivara

O. sativa japonica

Diversity in closest wild progenitor species

…Knowing genetic structure of wild accessions helps in the choice of parents

Mapping yield enhancing QTLs from O. *rufipogon*

IR58025A x KMR3

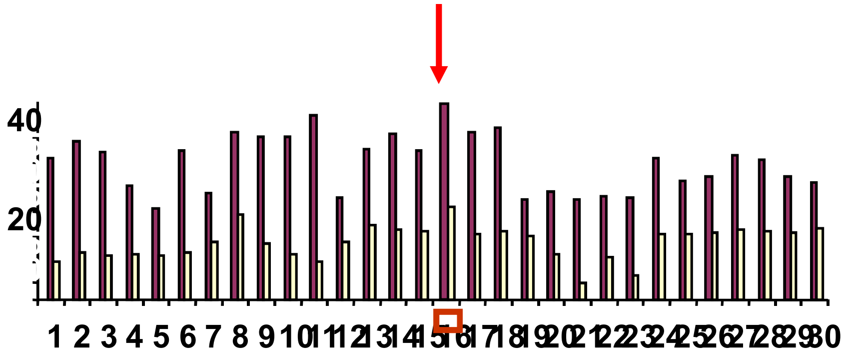
Karnataka Rice Hybrid 2

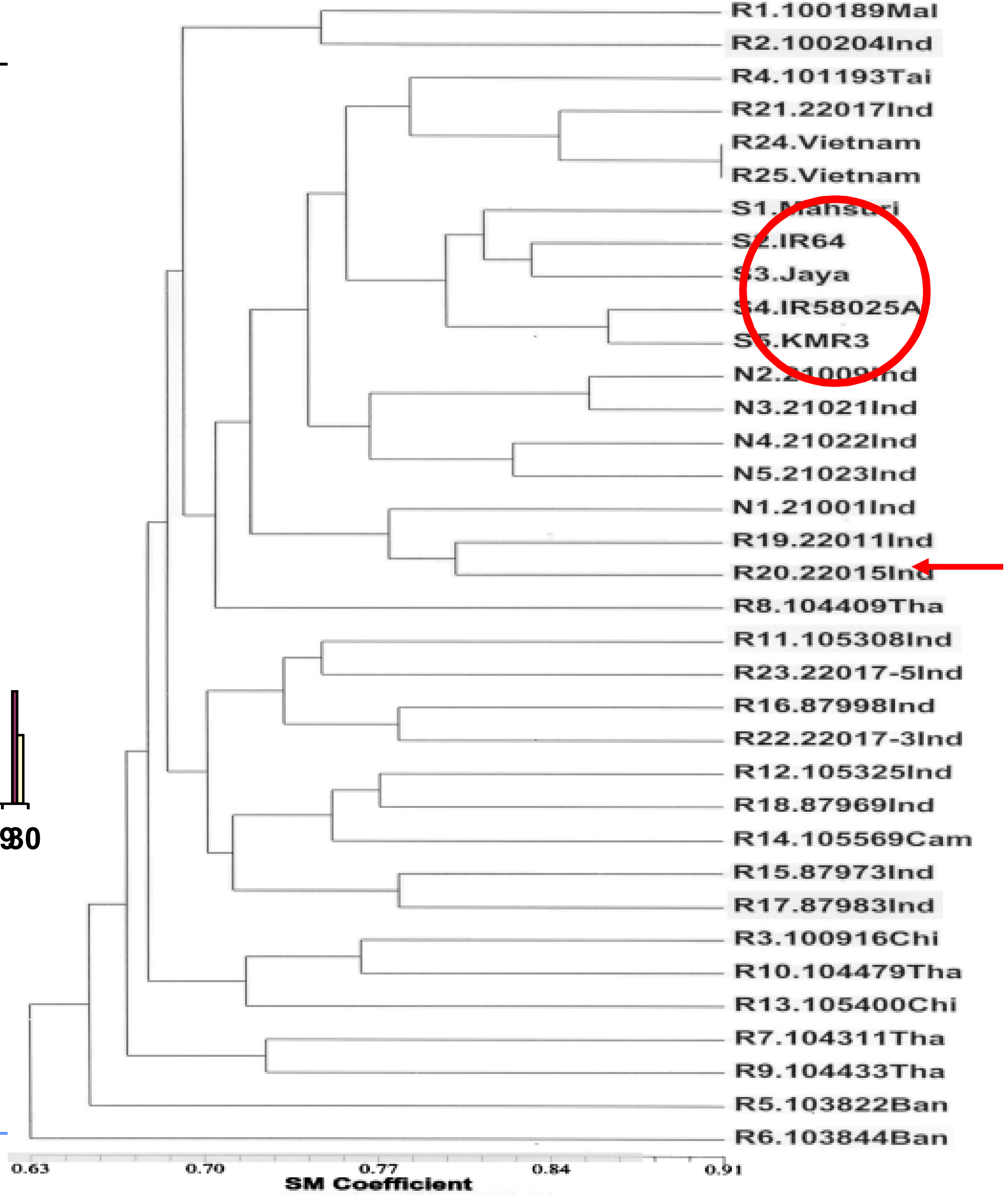
Which accession of wild species can be a donor for high yield ?

Diversity in *O. rufipogon*

Accession IC22015 (R20) identified as a donor

Accessions very distant (0.36-0.40) or very close (0.21-0.25) produced less vigorous hybrids





Advanced backcross method for QTL detection

(A) **IR 58025 A** **X** *O. rufipogon* accession (Kharif'99)

IC 22015

F1 **X** **IR 58025 B** (Rabi'00)

BC1 **X** **IR 58025 B** (Kharif'00)

(Select 50 plants)

BC2 **X** **Restorer (KMR3)** (Rabi'01)

(Select 251 plants)

251 BC2 test cross families (Kharif'01)

Phenotype for yield and associated traits

Genotype for segregation of each polymorphic molecular marker

(B) **IR 58025 A** **X** **Restorer (KMR3)**

Hybrid (KRH 2)

Phenotype for yield and associated traits

Advanced backcross method

wild progenitor species are easy to cross with cultivars

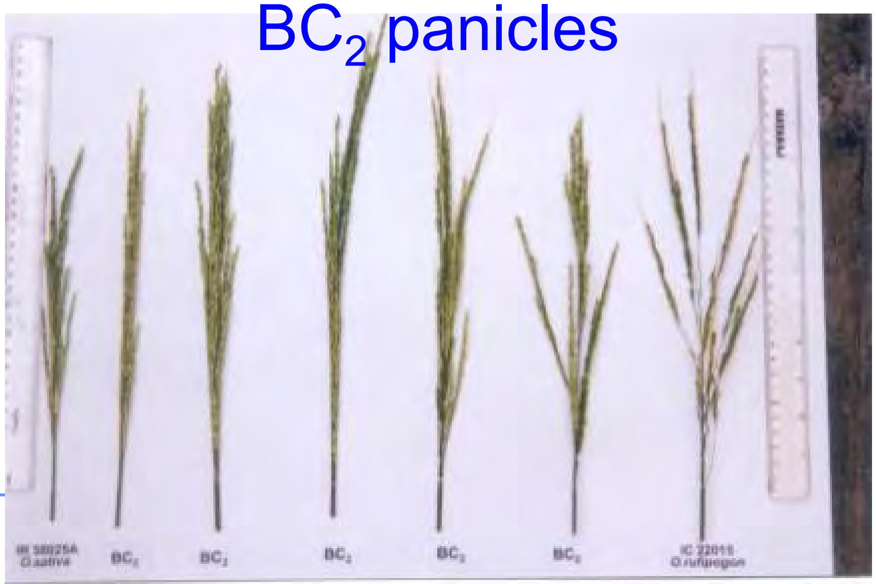
IR 58025A, *O.sativa*

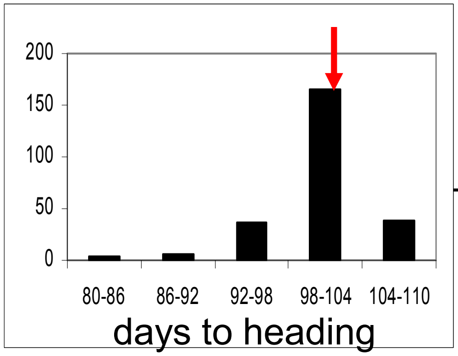
F_1: IR 58025A x IC 22015

IC 22015, *O.rufipogon*

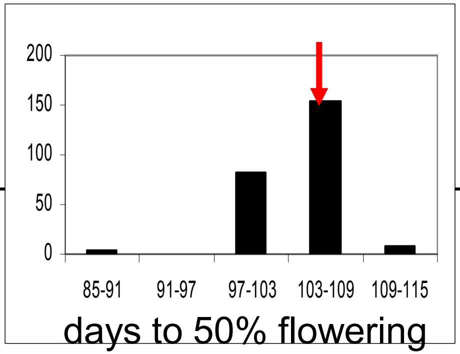
BC$_2$ plants

BC$_2$ panicles

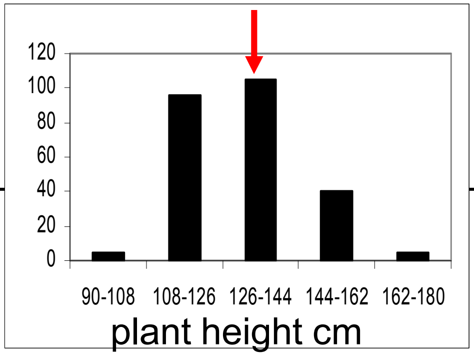




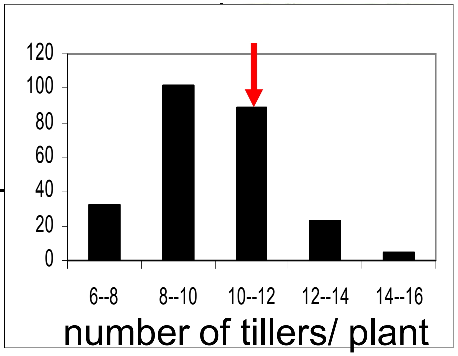
days to heading



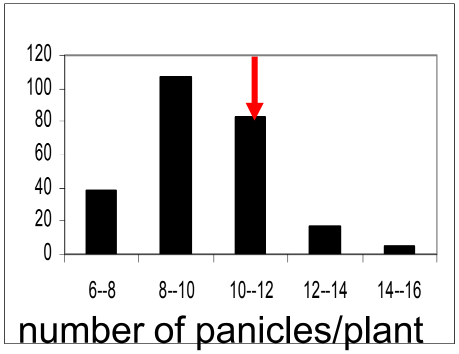
days to 50% flowering



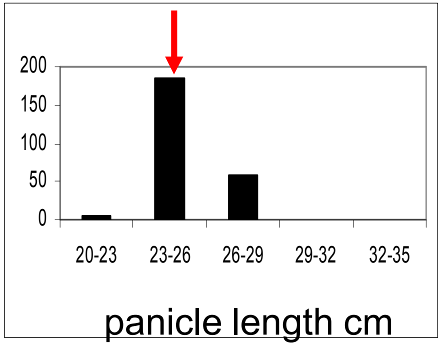
plant height cm



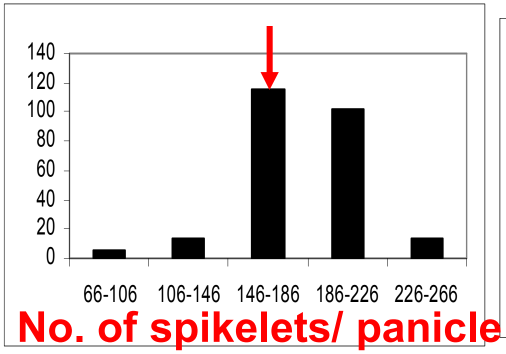
number of tillers/ plant



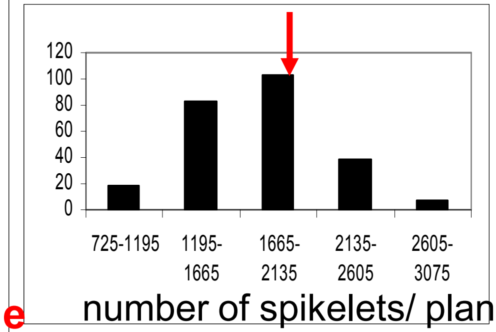
number of panicles/plant



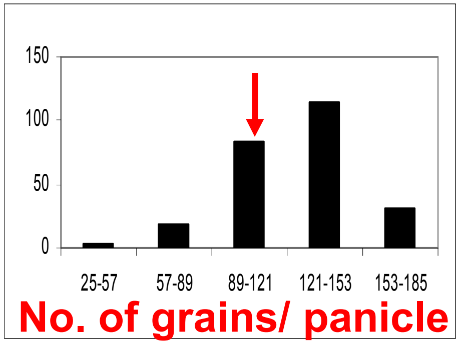
panicle length cm



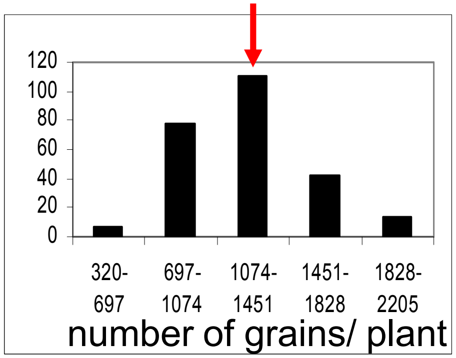
No. of spikelets/ panicle



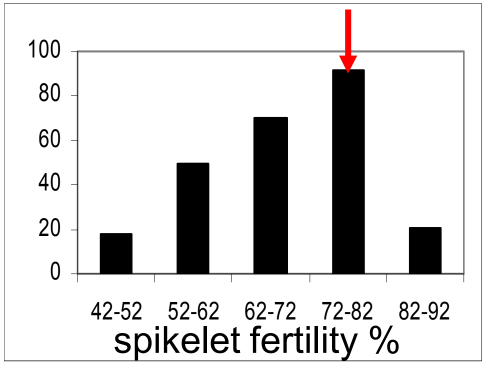
number of spikelets/ plan



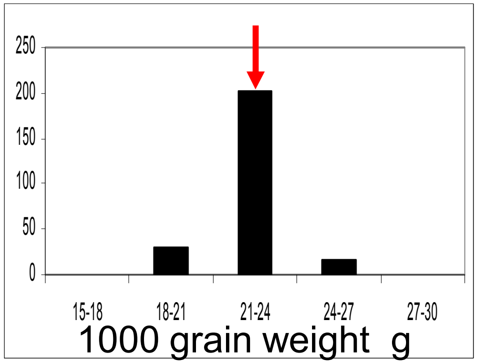
No. of grains/ panicle



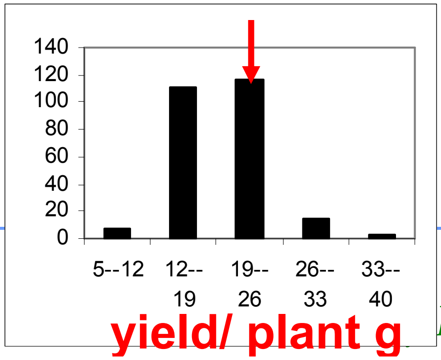
number of grains/ plant



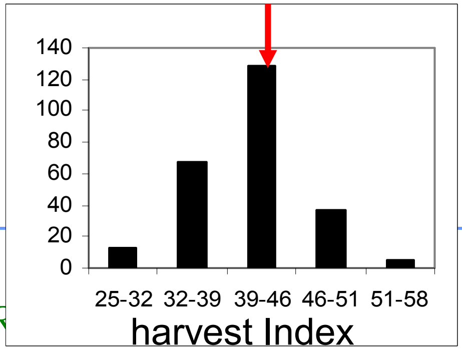
spikelet fertility %



1000 grain weight g



yield/ plant g

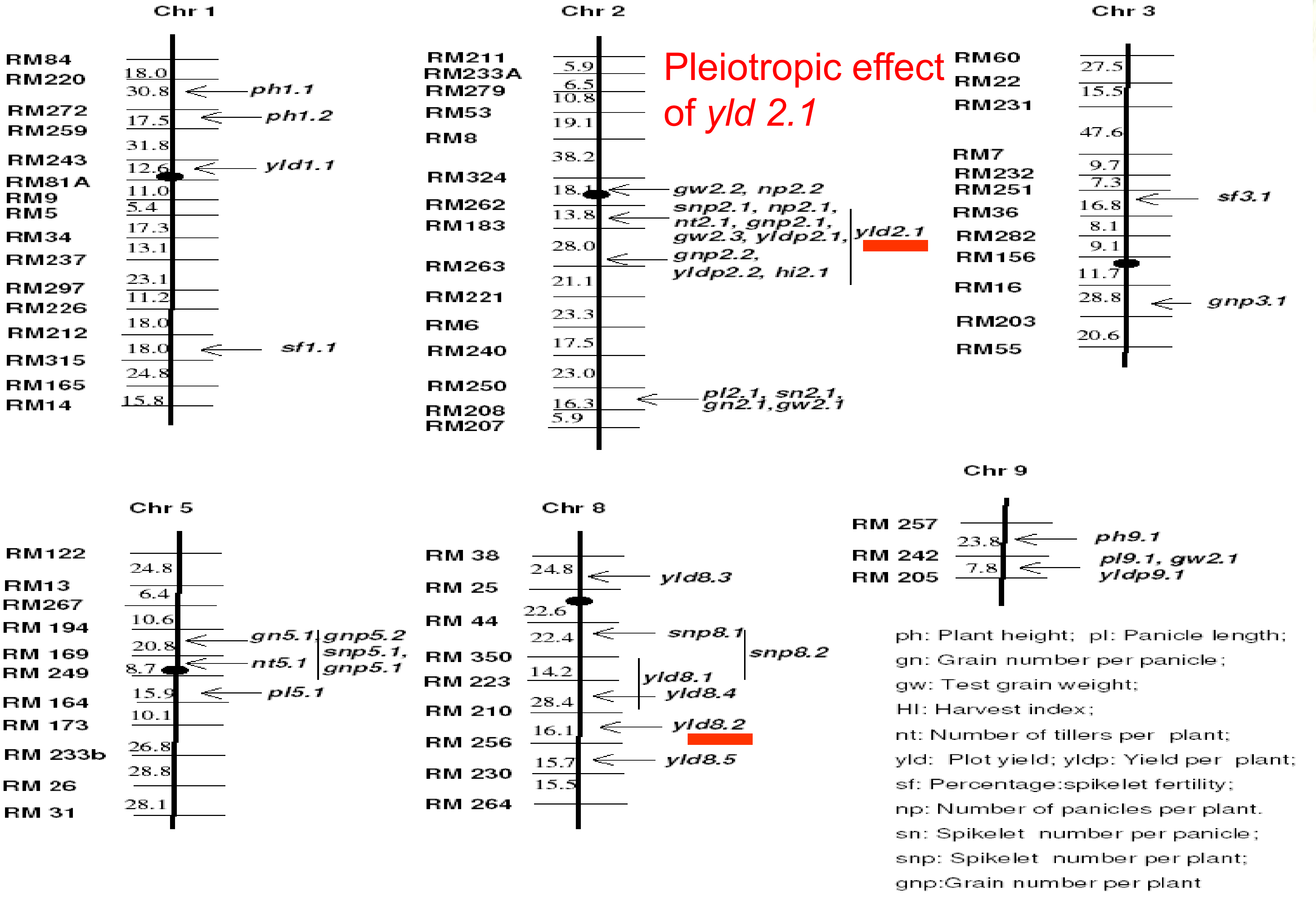


harvest Index

Frequency distribution of 251 families

↓ KRH2 value

Pleiotropic effect of *yld 2.1*



ph: Plant height; pl: Panicle length;
gn: Grain number per panicle;
gw: Test grain weight;
HI: Harvest index;
nt: Number of tillers per plant;
yld: Plot yield; yldp: Yield per plant;
sf: Percentage:spikelet fertility;
np: Number of panicles per plant.
sn: Spikelet number per panicle;
snp: Spikelet number per plant;
gnp:Grain number per plant

Figure 2: Distribution of QTLs on t
on BC_2 testcross po

Marri et al 2005 BMC Genetics 6, 33

Two yield enhancing QTLs from *O. rufipogon* identified on chromosome 2 and 8

BMC Genetics

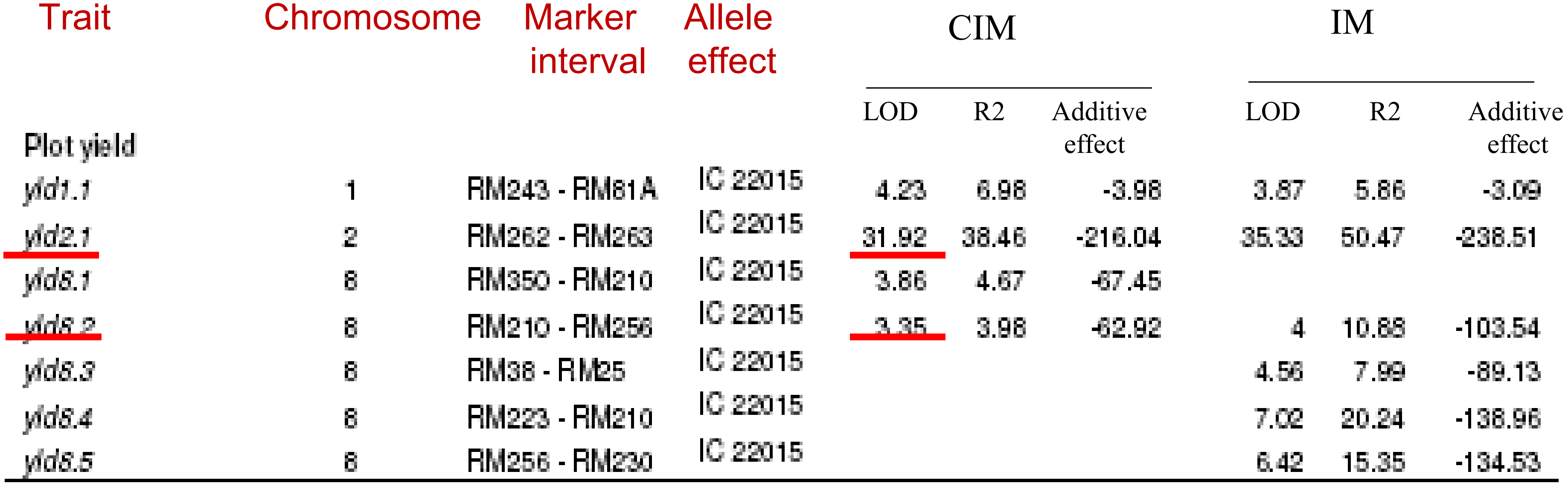
BioMed Central

Research article

Open Access

Identification and mapping of yield and yield related QTLs from an Indian accession of *Oryza rufipogon*

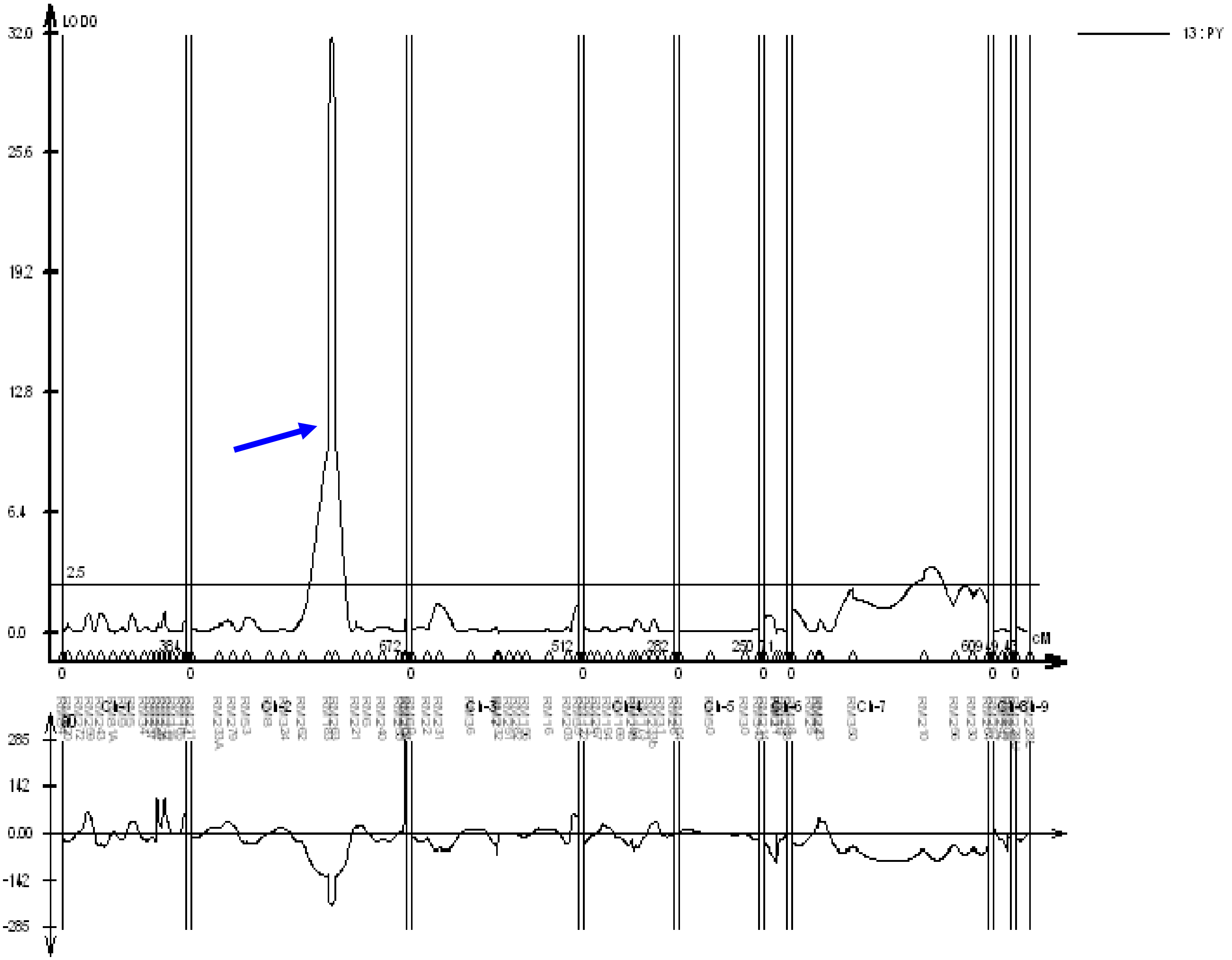
Pradeep Reddy Marri*, Sarla N, Laxminarayana V Reddy and EA Siddiq



Trait	Chromosome	Marker interval	Allele effect	CIM			IM		
				LOD	R2	Additive effect	LOD	R2	Additive effect
Plot yield									
yld1.1	1	RM243 - RM81A	IC 22015	4.23	6.98	-3.98	3.87	5.86	-3.09
yld2.1	2	RM262 - RM263	IC 22015	31.92	38.46	-216.04	35.33	50.47	-238.51
yld8.1	8	RM350 - RM210	IC 22015	3.86	4.67	-67.45			
yld8.2	8	RM210 - RM256	IC 22015	3.35	3.98	-62.92	4	10.88	-103.54
yld8.3	8	RM38 - RM25	IC 22015				4.56	7.99	-89.13
yld8.4	8	RM223 - RM210	IC 22015				7.02	20.24	-138.96
yld8.5	8	RM256 - RM230	IC 22015				6.42	15.35	-134.53

Xiao et al 1998 Genetics *yld2.1* is more distal at RM250-RM208

One major effect QTL on chromosome 2



Beyond QTL mapping

- Marker assisted introgression

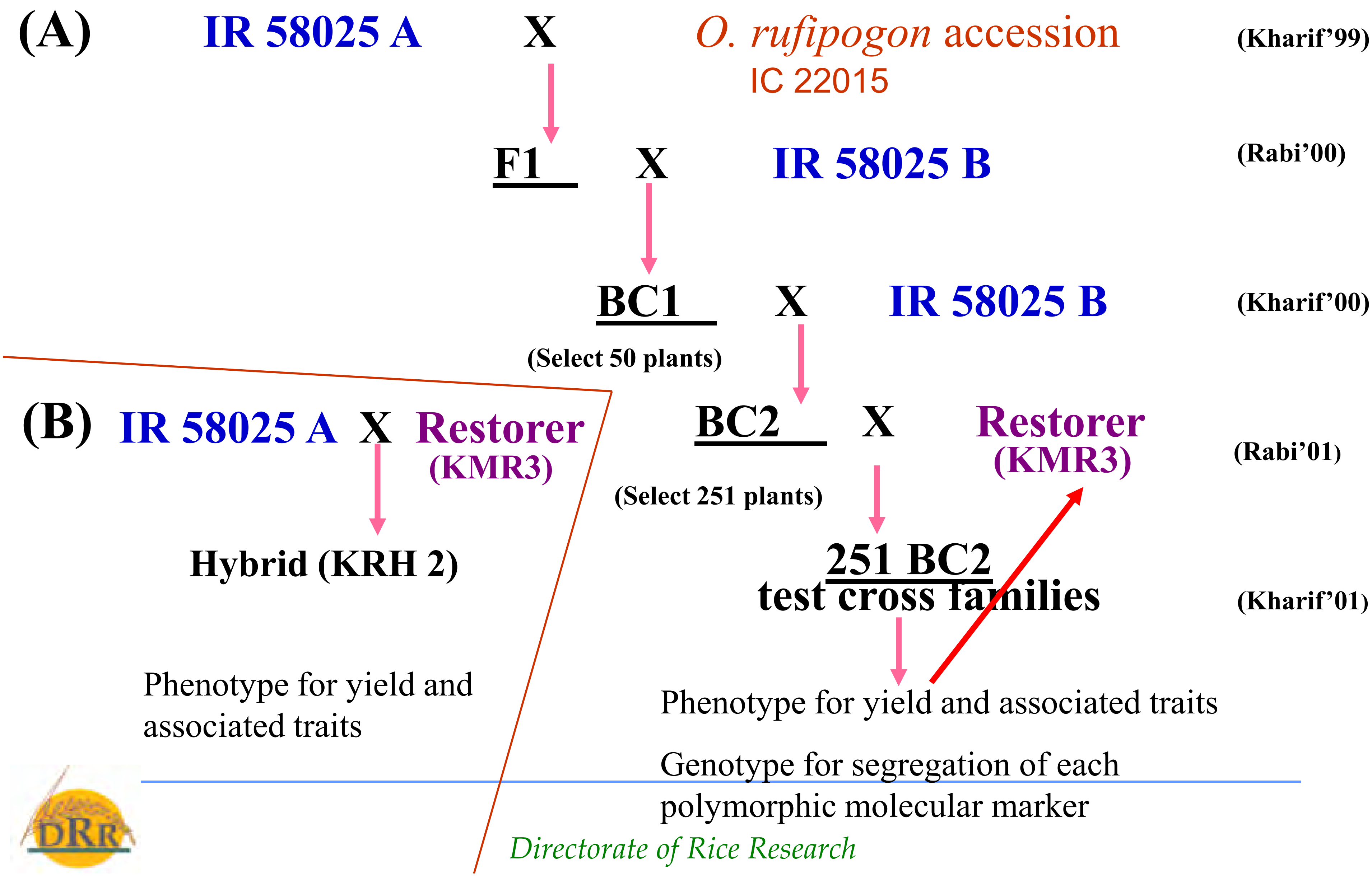
  - Improving yield of KMR3, the restorer for release as a variety

  - Improving yield of derived hybrids

  - developing QTL-NIL for fine mapping

- Fine mapping for gene discovery
  (*in progress in collaboration with Dr Rajeshwari Ramanan, CCMB*)

Advanced backcross method for QTL detection



(A) **IR 58025 A** **X** *O. rufipogon* accession IC 22015 **(Kharif'99)**

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Phenotype for yield and associated traits

Genotype for segregation of each polymorphic molecular marker

(B) **IR 58025 A** **X** **Restorer (KMR3)**

Hybrid (KRH 2)

Phenotype for yield and associated traits

Introgression lines of KMR3-*O.rufipogon*

KMR3 X Test cross progeny P105, P26 with *yld2.1*

↓

KMR3 X BC1F1 (215 plants)

↓

KMR3 X BC2F1 (406 plants)

↓

BC_3F_1 (504 plants analyzed with 8 sub QTL markers)

↓ X

BC_3F_2 (960 plants, those which had either 1, 2 or 3 continuous markers identified)

↓ X

BC_3F_3 (55 families with 1, 2 or 3 continuous markers)

Marker assisted backcrosses using flanking markers (30%)

APrasad Babu, PhD thesis, 2010

8 SSR markers polymorphic within *yld2.1* RM262 - RM 263 in 504 BC_3F_1 plants (only 10/84 SSRs polymorphic)

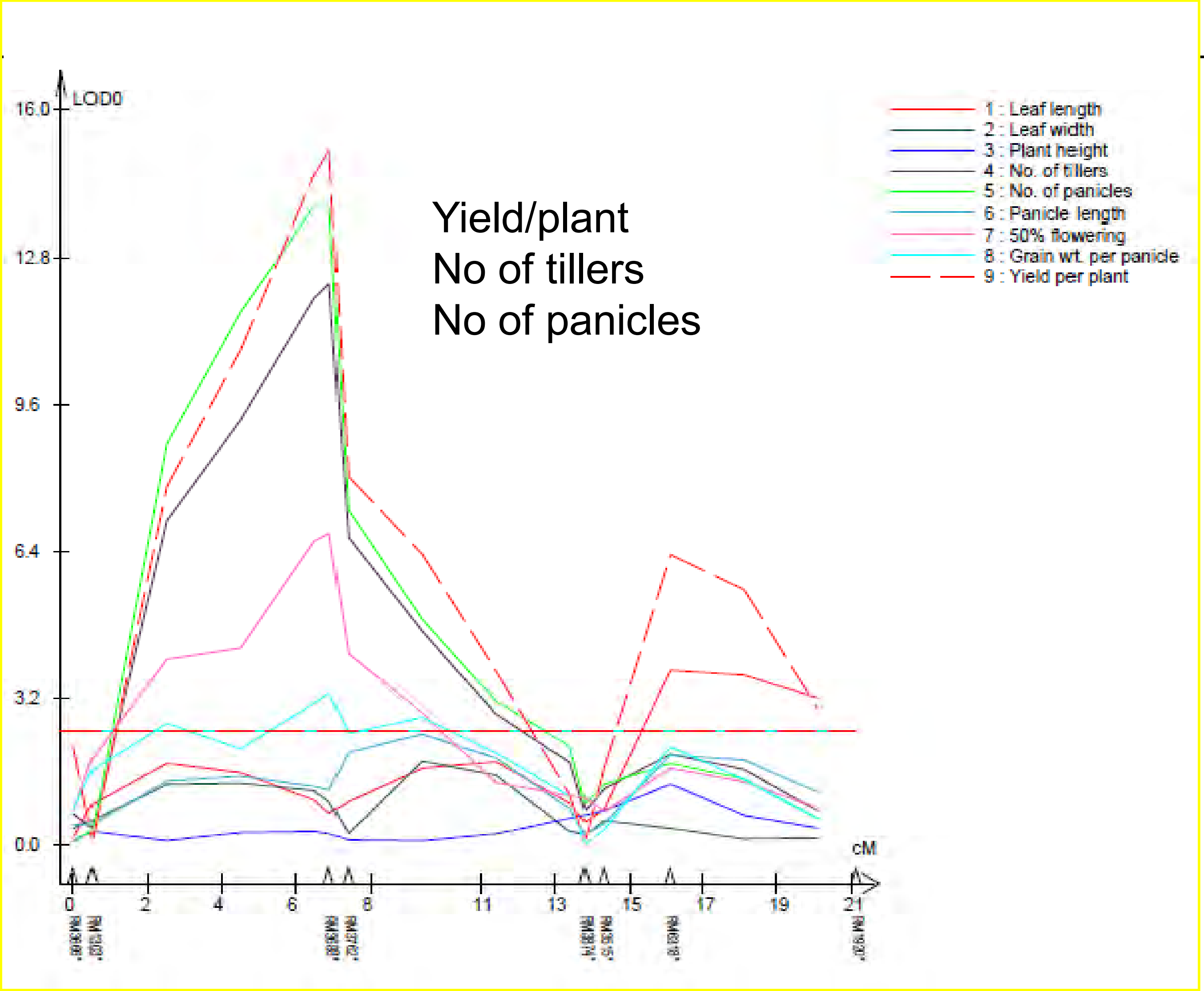
RM 262

RM 3666	1- 38
RM 1303	14 -170
RM 3688	1 - 93
RM 3762	54 - 235
RM 3874	12 - 130
RM 3515	1 - 147
RM 6318	4 - 164
RM 1920	19 - 207

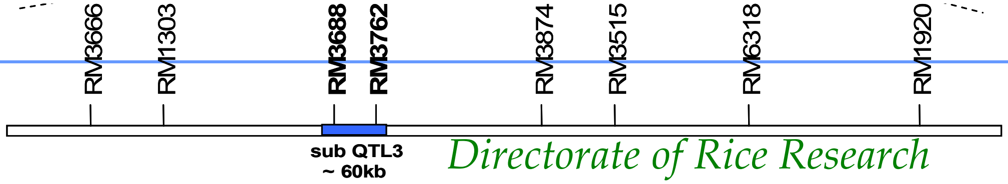
RM 263

106

Mapping of 9 traits to sub QTL 3 (62 Kb) of *yld2.1*

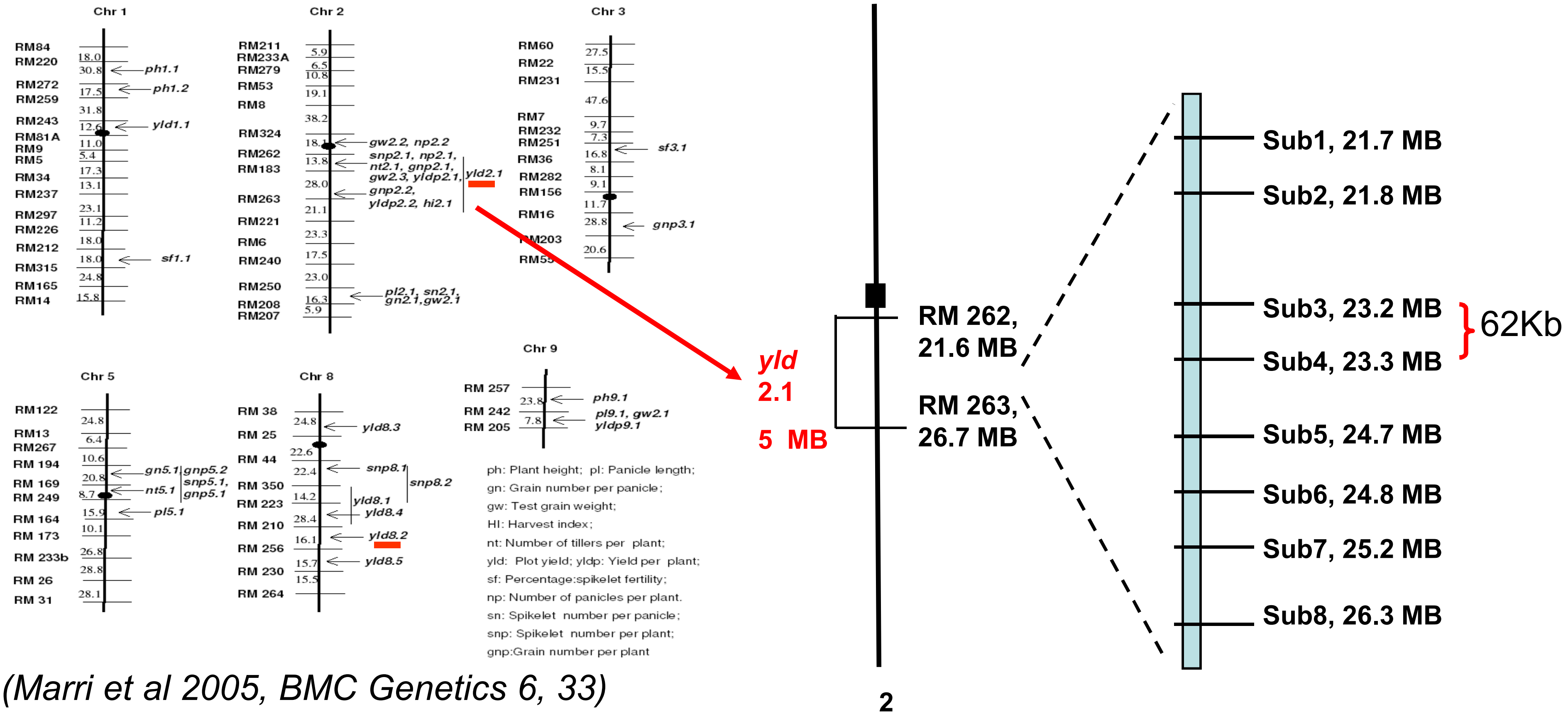


Yield/plant
No of tillers
No of panicles



KMR3-*O.rufipogon*
yld2.1 narrowed to one sub QTL

MABC for 3 generations, marker analysis of 504 BC3F1 plants and derived BC3F2



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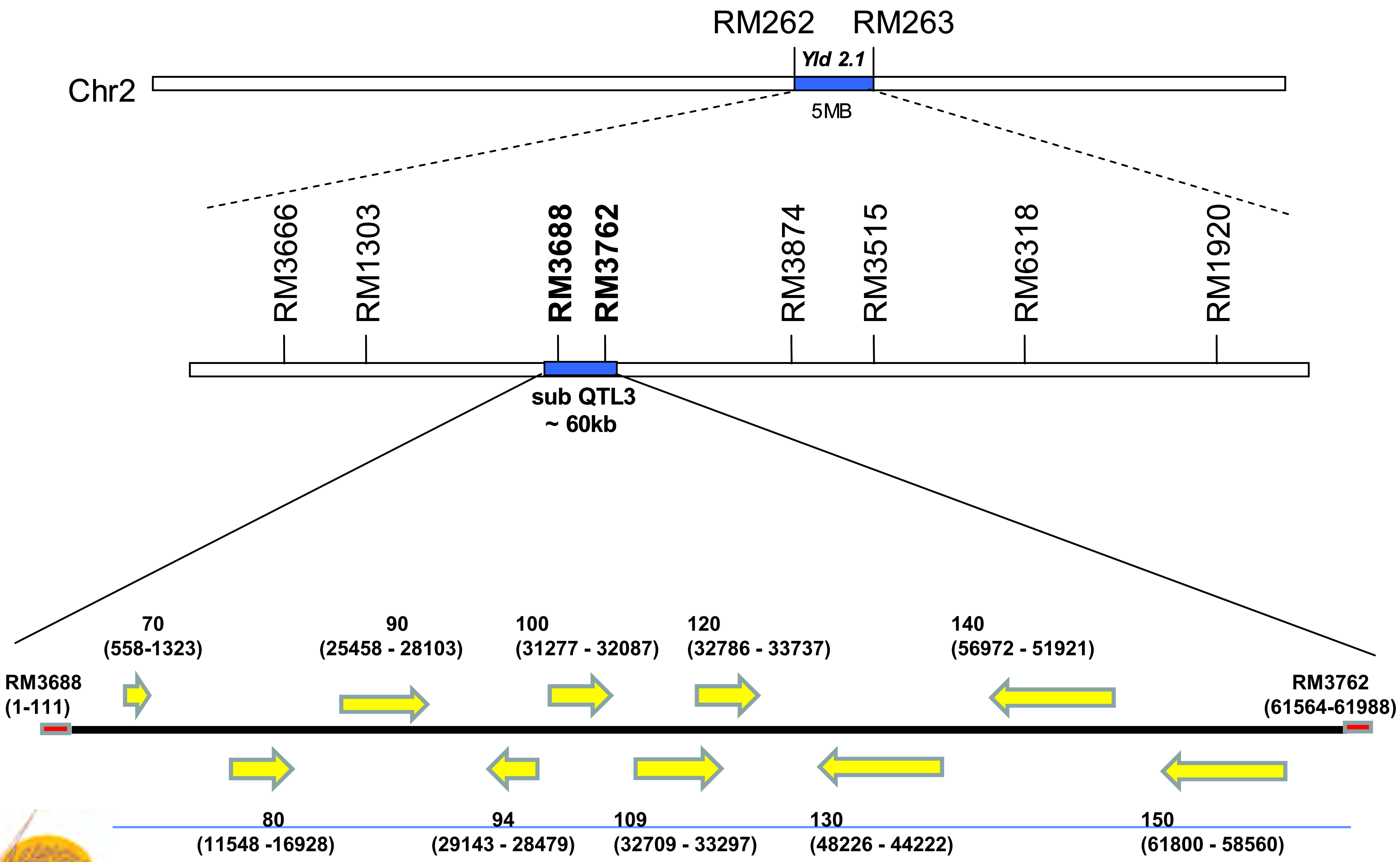
(Marri et al 2005, BMC Genetics 6, 33)

Candidate genes in sub QTL3 of *yld2.1*



RM262 RM263

Yld 2.1

Chr2

5MB

RM3666 RM1303 **RM3688** **RM3762** RM3874 RM3515 RM6318 RM1920

sub QTL3
~ 60kb

RM3688
(1-111)

70
(558-1323)

90
(25458 - 28103)

100
(31277 - 32087)

120
(32786 - 33737)

140
(56972 - 51921)

RM3762
(61564-61988)

80
(11548 -16928)

94
(29143 - 28479)

109
(32709 - 33297)

130
(48226 - 44222)

150
(61800 - 58560)

Field evaluation of introgression lines

Sarla et al 2009. DRR Newsletter 7(2),9

IL	Yield kg/10sqm plot
S-50	9.70
S-458	8.90
S-215	8.02
S-463	7.72
S-194	7.70
S-495	7.68
S-467	7.68
S-501	7.41
S-106	7.23
KMR-3	7.20

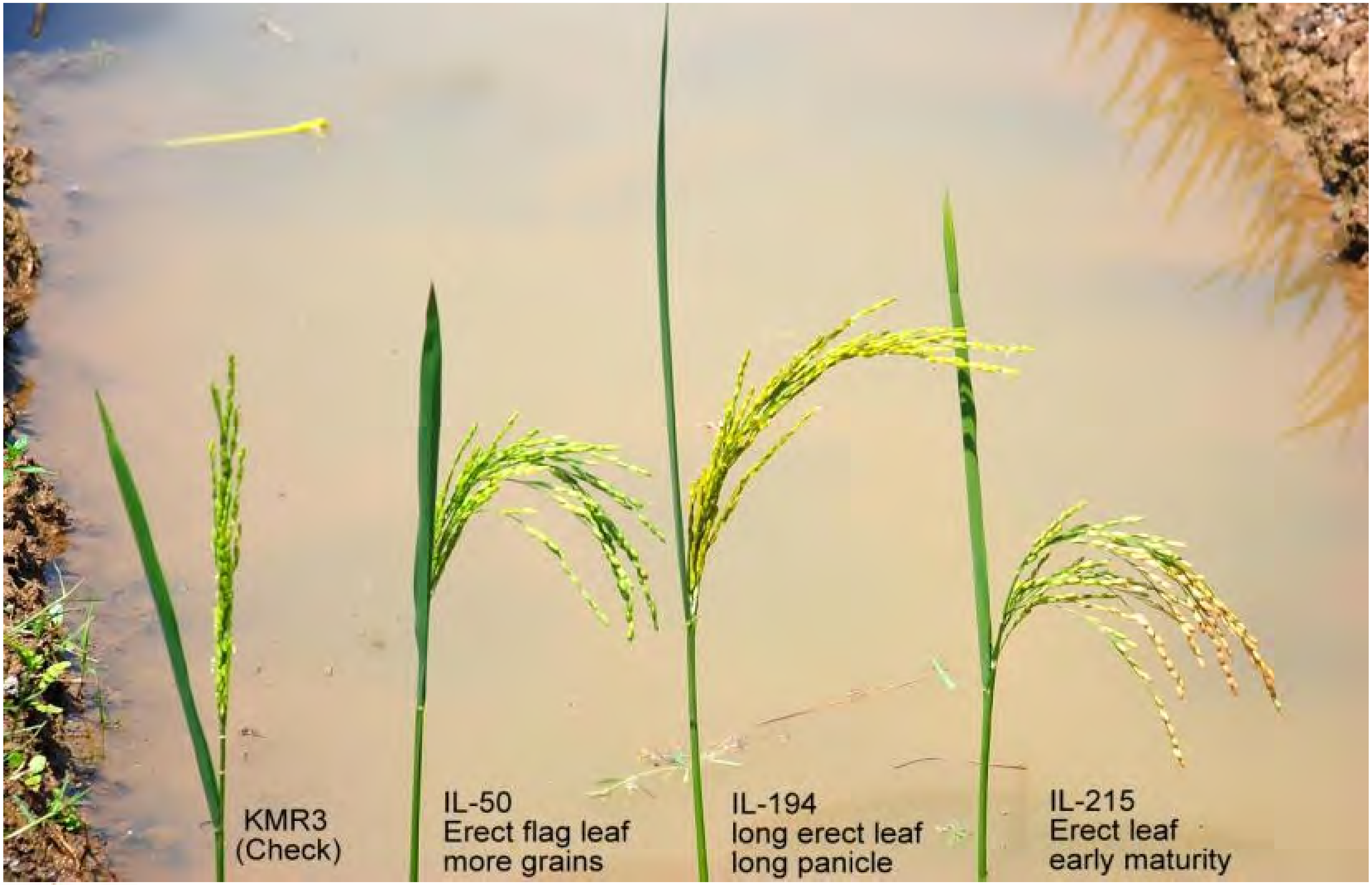
High yielding introgression line

KMR3

KMR3 IL 50

ILs mature earlier than KMR3

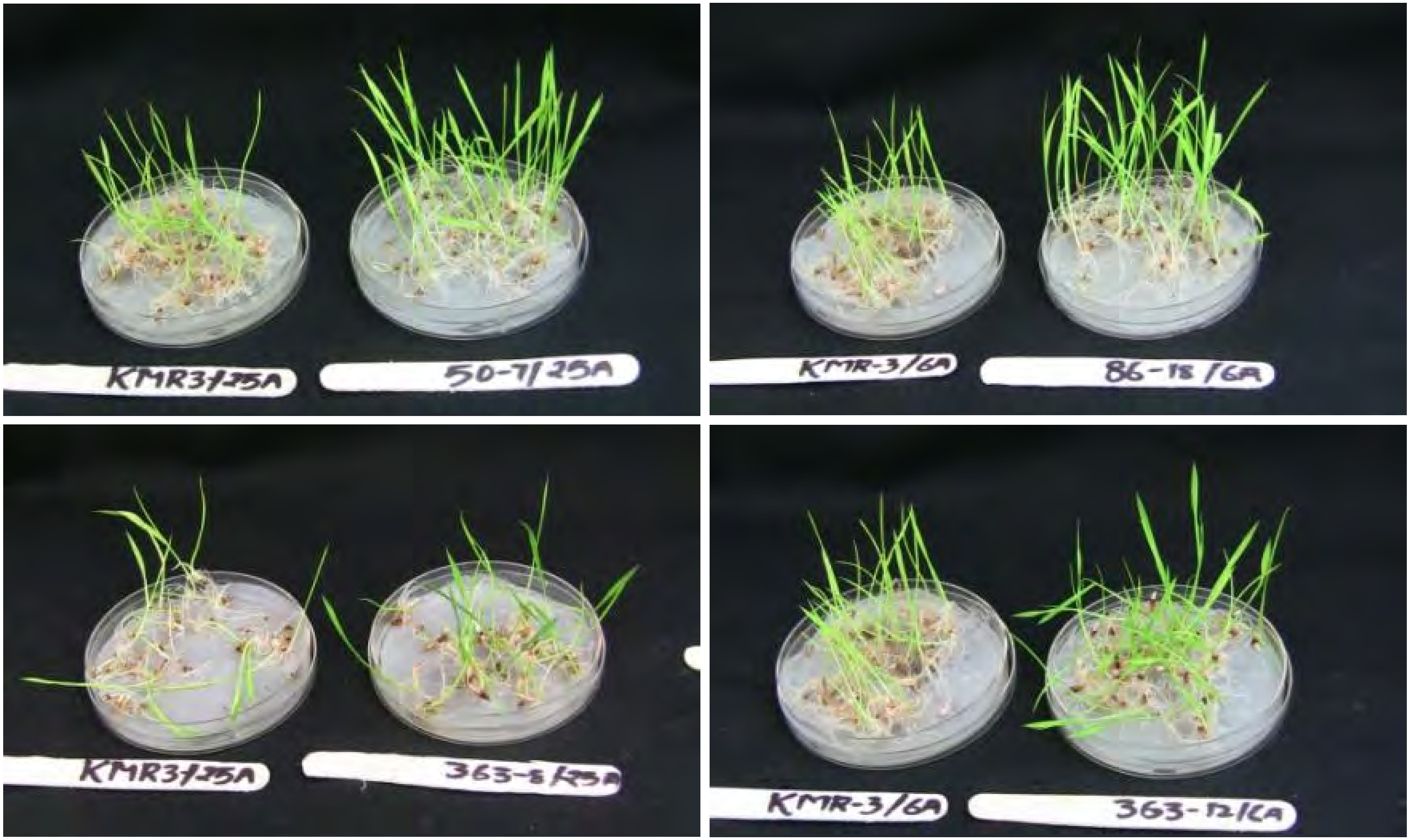
Variation in flag leaf and panicle in ILs



Increased yield in KMR3 - *O. rufipogon* ILs

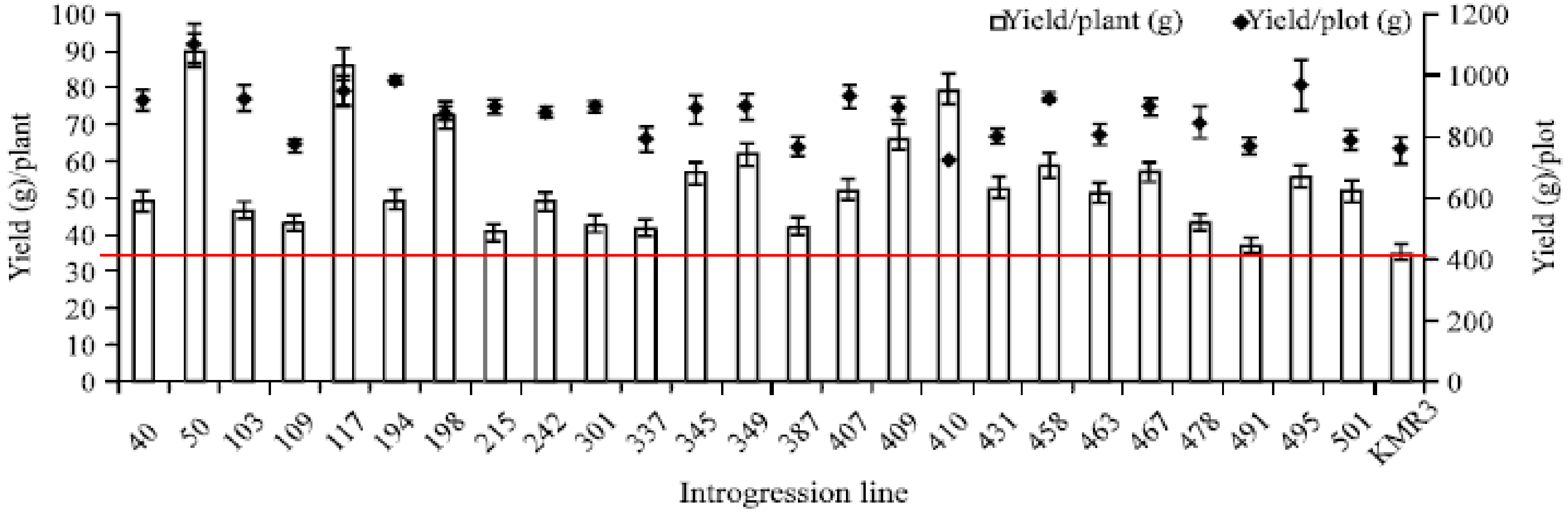
KMR3 ILs /Checks	Plant height (cm)	Days to maturity	Plot yield (g)	% yield increase over KMR3	% yield increase over Annada
RPBio4919-50	151	126	1101	45.6	27.3
RPBio4919-194	163	124	982	29.9	13.5
RPBio4919-495	141	132	967	27.9	11.8
RPBio4919-117	141	127	946	25.1	9.4
RPBio4919-106	137	130	935	23.7	8.1
RPBio4919- 458	143	125	924	22.2	6.8
RPBio4919-349	117	133	896	18.5	3.6
RPBio4919-467	140	126	895	18.4	3.5
RPBio4919-215	105	130	895	18.4	3.5
RPBio4919-198	142	125	875	15.7	1.2
RPBio4919-463	143	124	805	6.5	-
RPBio4919-501	153	124	785	3.8	-
KMR3 (Check-1)	149	135	756	0	
Annada (Check-2)	94	122	865	11.4	Best check
IR64 (Check-3)	99	124	842	14.4	

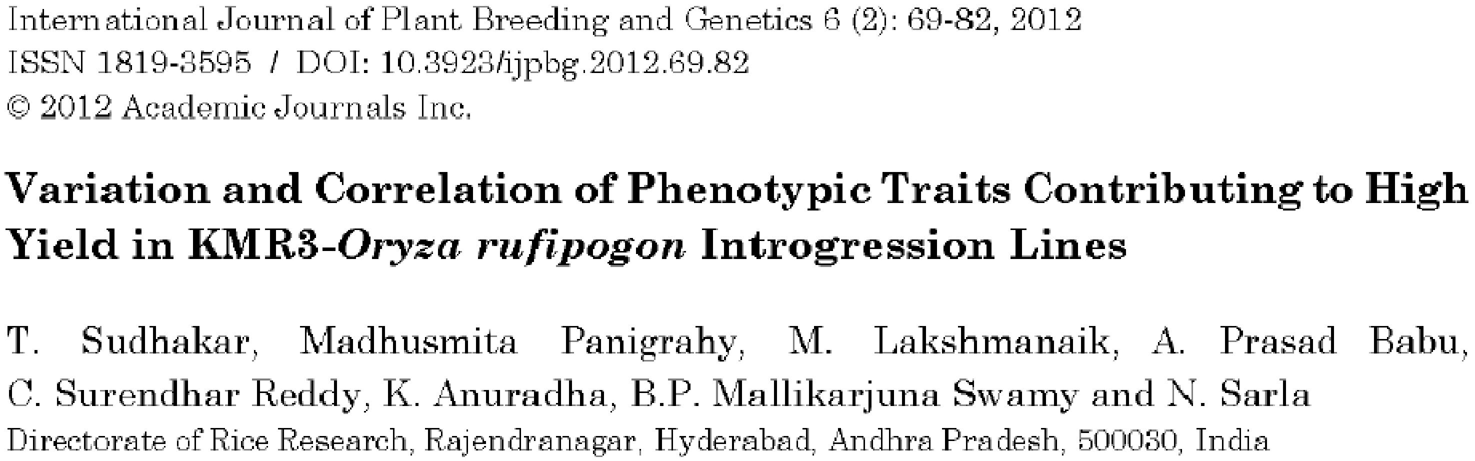
High initial vigor in hybrids derived using different cms lines and KMR3 ILs with sub QTL3



25 high yielding KMR3 ILs analysed for yield related traits

Int. J. Plant Breed. Genet., 6 (2): 69-82, 2012



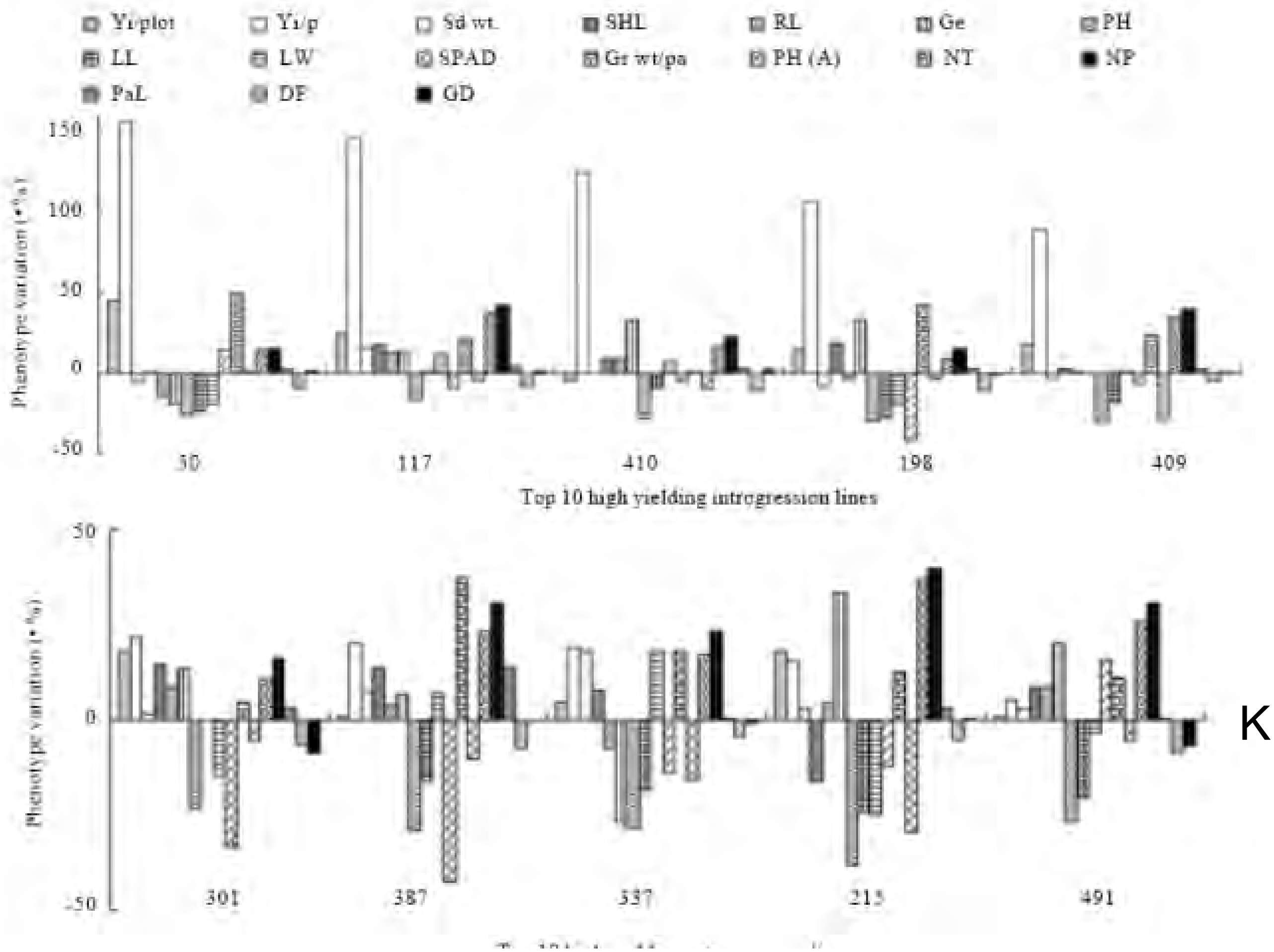

International Journal of Plant Breeding and Genetics 6 (2): 69-82, 2012
ISSN 1819-3595 / DOI: 10.3923/ijpbg.2012.69.82

Variation and Correlation of Phenotypic Traits Contributing to High Yield in KMR3-*Oryza rufipogon* Introgression Lines

T. Sudhakar, Madhusmita Panigrahy, M. Lakshmanaik, A. Prasad Babu, C. Surendhar Reddy, K. Anuradha, B.P. Mallikarjuna Swamy and N. Sarla
Directorate of Rice Research, Rajendranagar, Hyderabad, Andhra Pradesh, 500030, India

Each IL has a different way of increasing yield

Int. J. Plant Breed. Genet., 6 (2): 69-82, 2012



KMR3

KMR3

Evaluation of KMR3 ILs for several traits

500 BC2F4 - 30 highest yielding lines selected for yield, used for hybrid rice development, and screening for stress tolerance

Abiotic stress

1. drought tolerance – Dr Vandana Rai, Dr T Ram
2. salinity tolerance – Dr Vandana Rai
3. direct seeded conditions – Dr Vandana Rai, Dr Suneetha
4. low sulphur field conditions – Dr Sridevi
5. low P field conditions – Dr Krishnamurthy, Dr Latha
6. SRI – Dr Mahendra Kumar
7. germination in submergence - Dr Vandana Rai

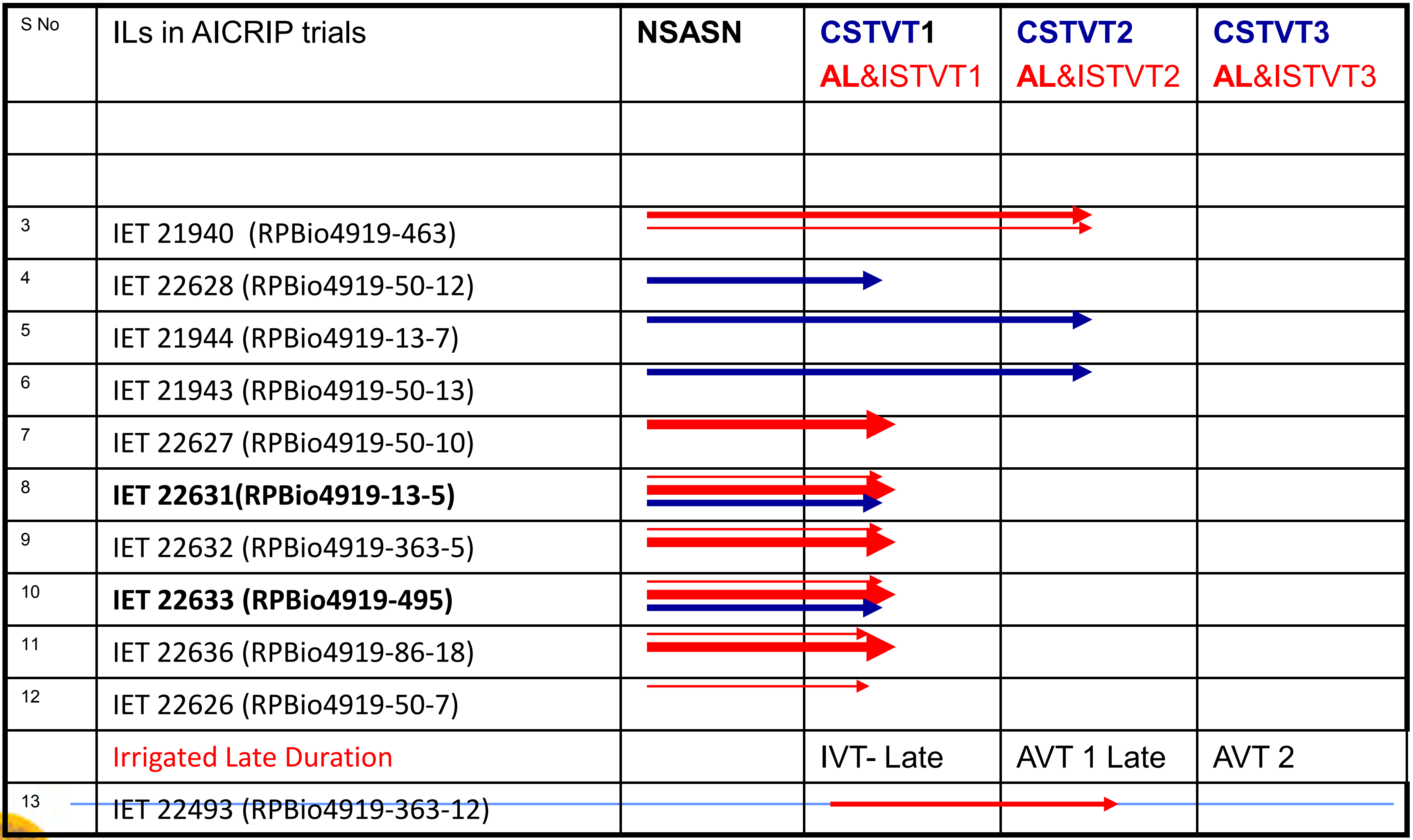
Biotic stress

1. BPH, WBPH tolerance – Dr Jhansi Lakshmi
2. Gall midge – Dr Bentur
3. Stem borer – Dr Padma Kumari
4. Blb resistance- Dr Laha
5. Blast resistance – Dr Srinivas Prasad, Dr Sheshu Madhav
6. Tungro – Dr Krishnaveni

Quality

1. grain quality- Dr N Shobna Rani

High yielding ILs in salinity and alkalinity trials



S No	ILs in AICRIP trials	**NSASN**	**CSTVT1** **AL&ISTVT1**	**CSTVT2** **AL&ISTVT2**	**CSTVT3** **AL&ISTVT3**
3	IET 21940 (RPBio4919-463)	→ →	→ →	→ →	
4	IET 22628 (RPBio4919-50-12)	→	→		
5	IET 21944 (RPBio4919-13-7)	→	→	→	
6	IET 21943 (RPBio4919-50-13)	→	→	→	
7	IET 22627 (RPBio4919-50-10)	→	→		
8	**IET 22631(RPBio4919-13-5)**	→ → →	→ → →		
9	IET 22632 (RPBio4919-363-5)	→ →	→ →		
10	**IET 22633 (RPBio4919-495)**	→ → →	→ → →		
11	IET 22636 (RPBio4919-86-18)	→ →	→ →		
12	IET 22626 (RPBio4919-50-7)	→	→		
	Irrigated Late Duration		IVT- Late	AVT 1 Late	AVT 2
13	IET 22493 (RPBio4919-363-12)		→	→	

% yield increase in SRI vs normal

	2009	2010
KMR3 – *O. rufipogon* ILs		
215	**25.49**	**15.04**
377-13	**40.28**	**15.74**
458	**23.65**	**26.73**

10 days early

root volume more

KMR3 - *O. rufipogon* ILs

- High yield
  irrigated, dry seeded, SRI
- High yield in hybrids
- Salinity tolerance
- Alkalinity tolerance
- Blb resistance
- Blast resistance

KMR3 - *O.rufipogon* IL as restorer for hybrid production

sub QTL3 from *O. rufipogon* is present in 7 of the top 11 hybrids out of 67 hybrids between 6 cms and 27 KMR3-ILs

	Hybrids	Yield/ plant (g)	Days to 50% fl	No of sec branches	Panicle wt (g)	*O. rufipogon* allele in KMR3 ILs
1	APMS6A * 363-12	**48.06**	71	47	3.82	**subQTL3**
2	CRMS32A * 50-10	**46.73**	74	51.6	4.66	RM 3762
3	APMS6A * 86-8	**44.8**	70	49.06	3.62	**subQTL3**
4	APMS6A*86-18	**43.80**	68	44.67	3.63	**subQTL3**
5	IR79156A*50-13	**43.47**	72	47.73	3.99	**subQTL3**
6	IR58025A*50-12	**42.47**	72	51.60	4.11	**subQTL3**
7	IR79156A*86-18	**42.33**	80	52.80	4.01	**subQTL3**
8	APMS6A * 410-5	**41.73**	69	53.53	3.96	RM 3688
9	APMS6A * 363-8	**40.26**	70	49.73	3.72	**subQTL3**
10	APMS6A * 91-15	**40.2**	75	62.46	3.67	RM 1303
11	CRMS32A * 410-5	**40**	68	46	3.66	RM 3688
	Checks					Heterozygous state
1	KRH2	28.3	79.0	46.0	3.4	
2	DRRH2	26.9	77.3	41.7	2.8	
3	**DRRH3**	**38.3**	**79.3**	**64.9**	**4.5**	
4	PA6201	28.9	79.3	41.3	2.7	
5	PA6444	26.8	75.0	46.6	3.0	
6	GK5003	30.1	71.3	41.9	3.4	

Fine mapping of sub QTL3 in progress in DBT project

Hybrids from ILs vs hybrids from KMR3 using 6 cms lines (g yield/ plant)

	Elite KMR3 ILs						
CMS/ R line	IL50-7	IL50-13	IL50-12	IL86-18	IL363-5	KMR3	F_1 Mean (L)
IR58025A	37.9	36.9	37.6	38.4*	30.5*	36.2	36.27
APMS6A	35.5**	35.9	32.8	43.4***	30.8	32.3	35.16
CRMS32A	42.4**	38.4	32.4*	38.0	31.4**	38.3	36.87
IR79156A	36.5	43.8	32.8*	42.3*	35.0	37.7	**38.04**
PUSA5A	33.0***	34.6**	33.5***	31.5***	32.1***	38.0	33.8
APMS10A	35.5*	34.3*	32.6	32.9	30.2*	32.4	33.01
F_1 Mean (T)	36.8	37.3	33.6	37.7	31.7	35.8	35.52

* $P<0.05$ ** P<0.01; *** P<0.001

negatively significant

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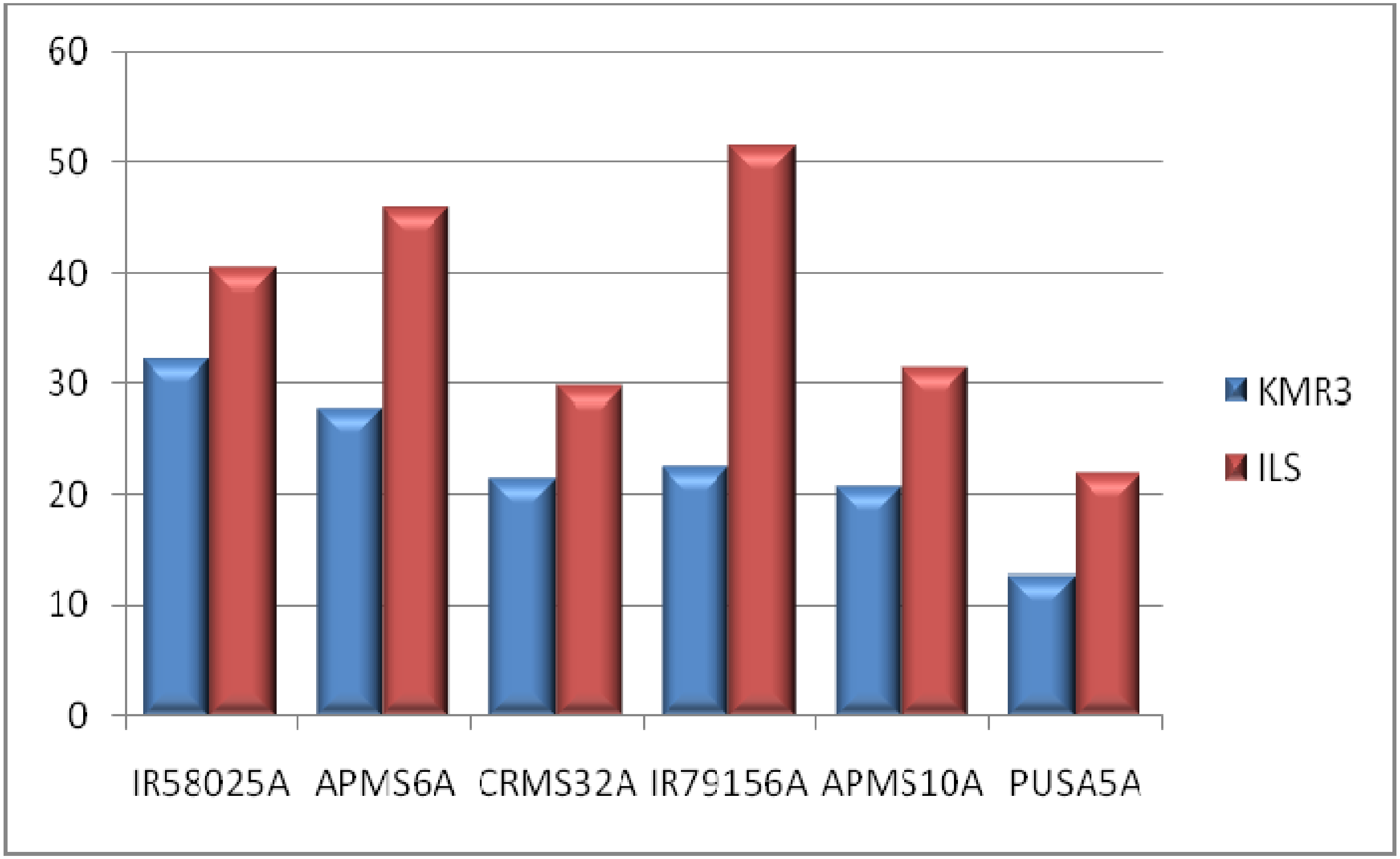
Higher standard heterosis in hybrids using KMR3-ILs



High standard heterosis in hybrids from ILs

CMS line	Mean Heterosis		Heterobeltiosis		Standard heterosis	
	KMR3	ILs	KMR3	ILs	KMR3	ILs
IR58025A	41.3	29.9- 84.9	25.7	14.5-77.4	12.2	**22.8-58.0**
APMS6A	23.5	23.2- 54.0	21.4	20.0-48.3	27.7	**28.7-63.0**
CRMS32A	27.0	24.0- 40.0	15.3	9.5-35.3	21.3	**20.6-39.0**
IR79156A	**33.8**	**26.0- 70.0**	**16.5**	**9.4-59.0**	**22.5**	**41.1-61.7**
APMS10A	18.8	17.0- 35.0	14.6	8.0-60.1	20.6	**20.3-42.6**
PUSA5A	33.5	15.0-54.4	11.5	0.4-31.4	12.6	**13.6-30.2**

Conclusions

- Genes from *rufipogon* help increase yield in restorer line KMR3
- Yield in ILs increased by different components
- Vigour and tolerance to abiotic and biotic stresses also increased
- Standarad heterosis is increased but in certain combinations

…..new genetic variation accelerates breeding for yield

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Thank you