

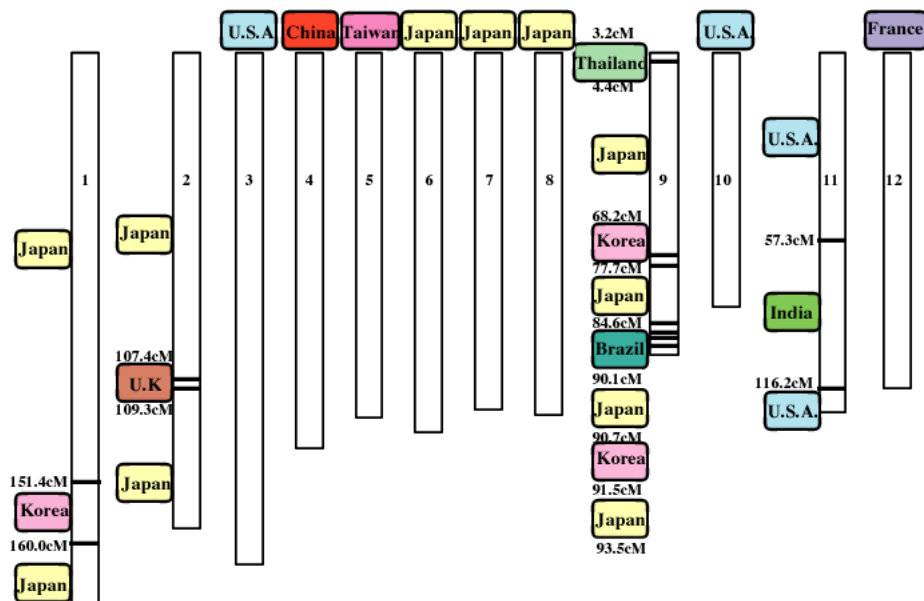
Crop Improvement by Allele Mining using the Map-Based Rice Genome Sequence

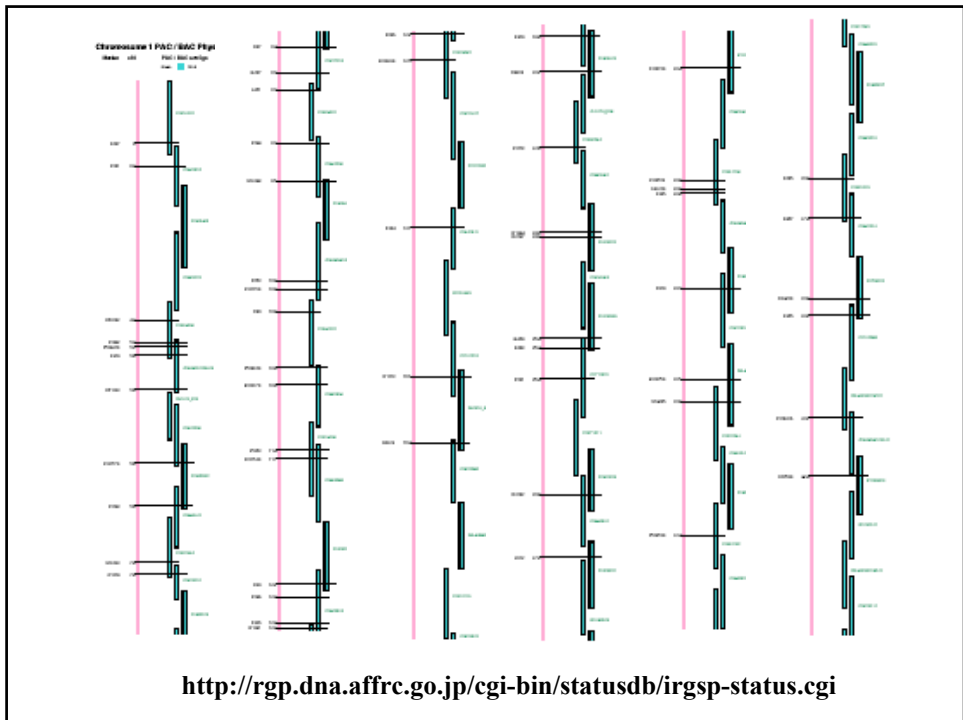
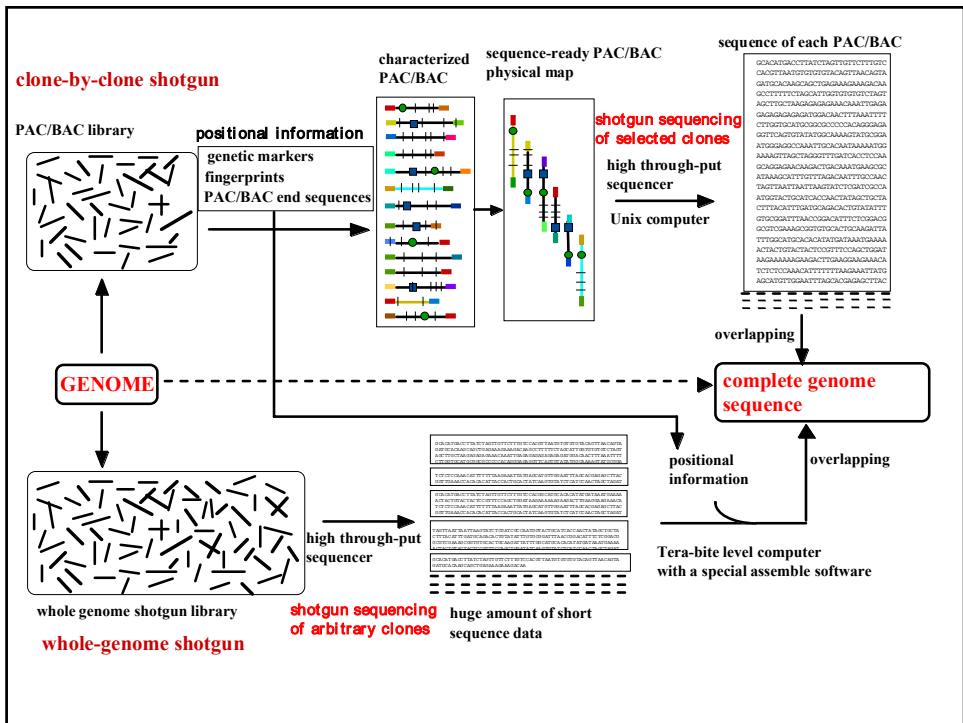
Takuji Sasaki

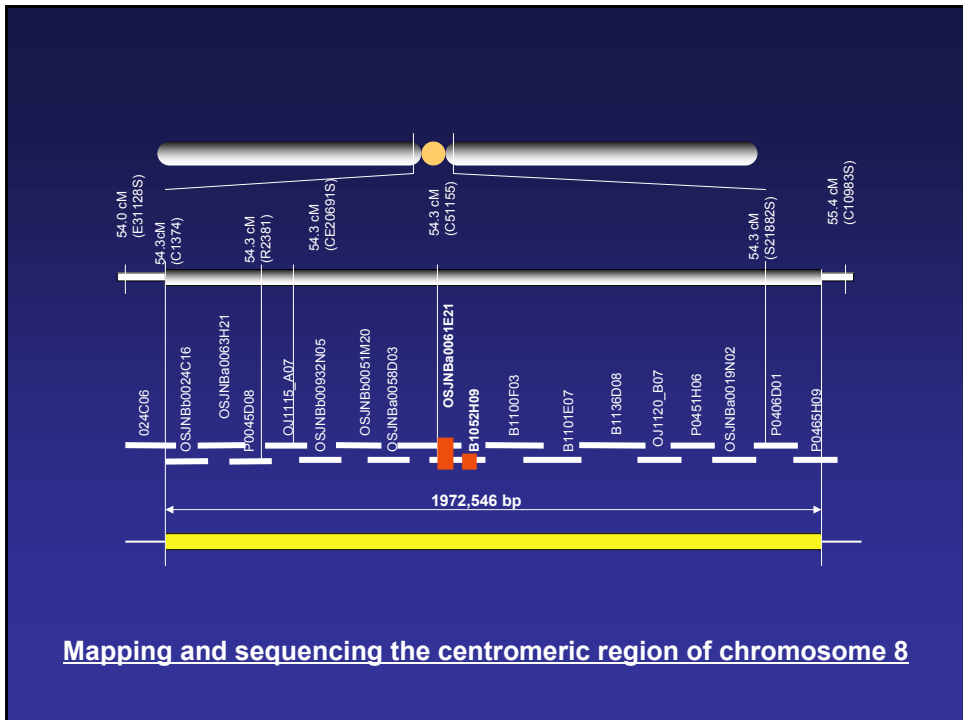
RGP, NIAS/STAFF



Chromosome sharing in IRGSP (1998-2004)







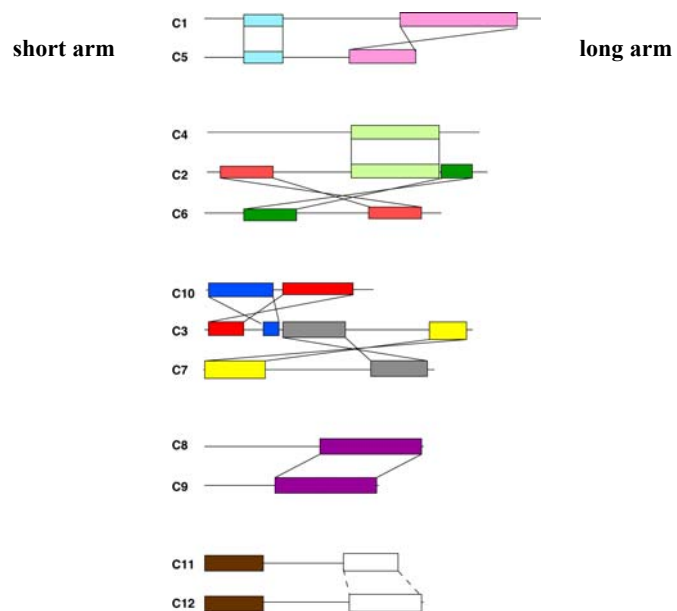
Mapping and sequencing the centromeric region of chromosome 8

total predicted gene number : 45,000

8.9 kb/gene



Major segmental duplications in the rice genome



Construction of Kasalath BAC physical maps with the tool of *in silico* mapping

Kasalath BAC library

Mbo I digest, 47,194 clones, insert size=133 kb.

Clone culture

96-well, two duplicates.

DNA isolation of BAC clones

Alkaline lysis (REAL Prep. Quadra3)

DNA sonication

About 1 kb in length (Sonifier 450)

BAC-end sequencing

ABI3700

Data trimming and quality check

E.coli, vector. Phred < 15

Filtration of repetitive sequences

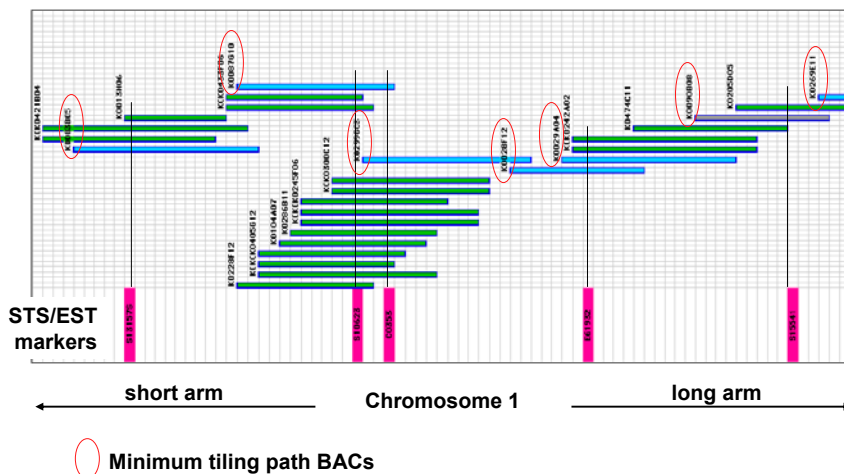
BLAST searching

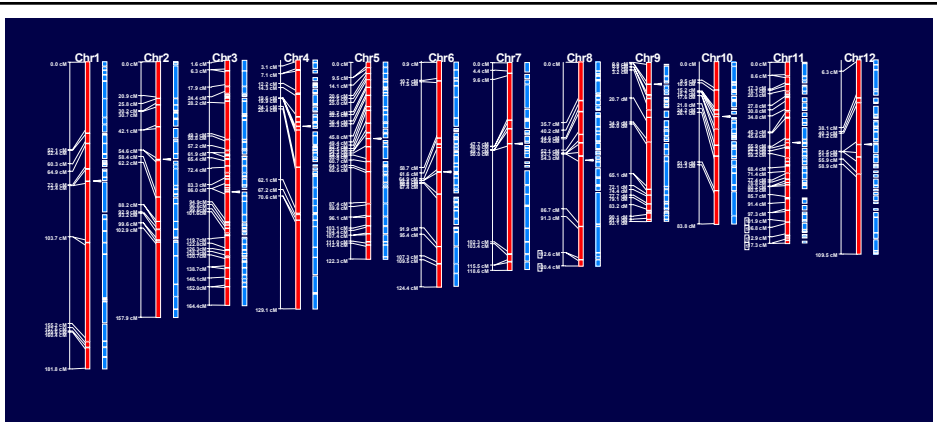
In silico chromosomal mapping

Sequence identity > 90% and alignment coverage > 50%; two mate-pairs < 200 kb apart; no ambiguity for orientation; BLAST Score E < 10⁻¹⁰⁰; a single hit, etc.

Map accuracy confirmed with DNA markers

Confirmation of *in silico* mapped BAC clones





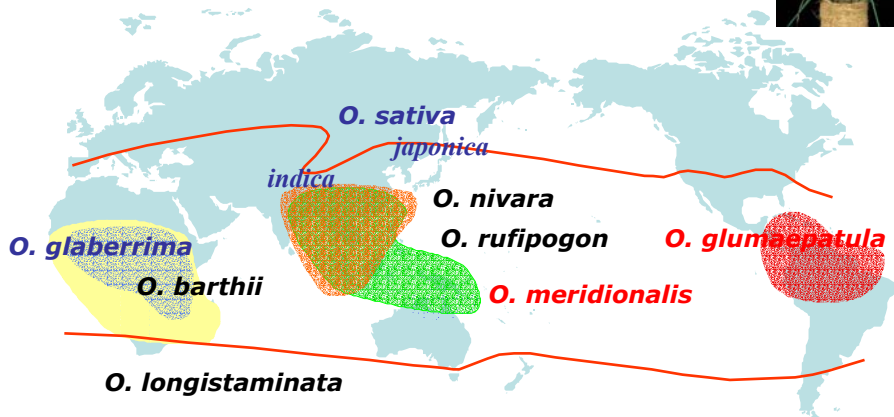
**Nipponbare
(japonica)
369Mb**

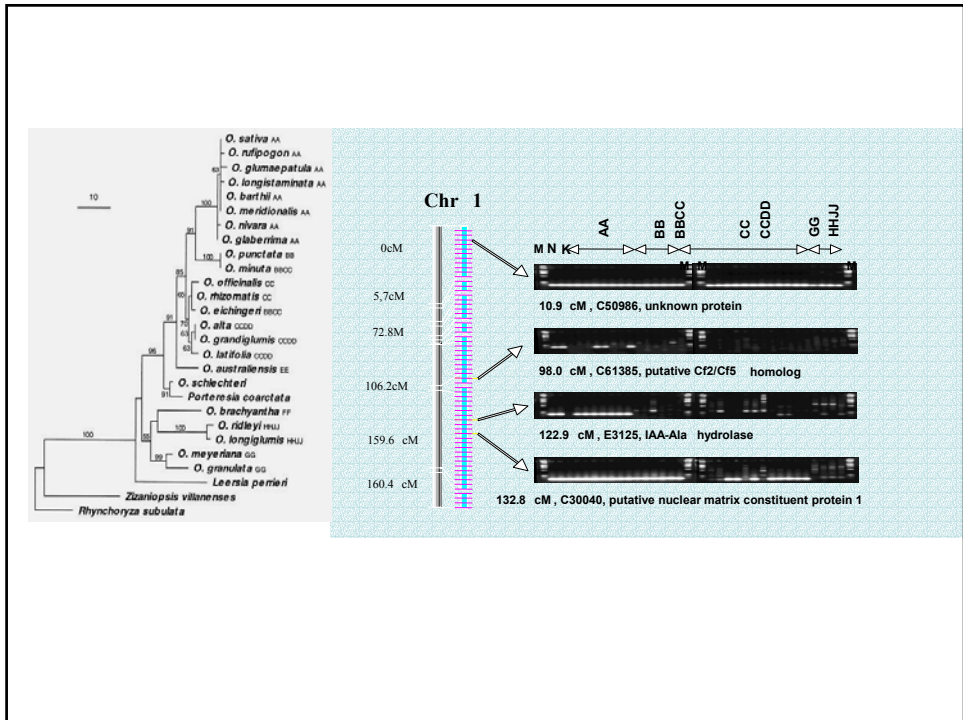


**Kasalath
(indica)
308Mb**

SNPs: every 100bp
In/Del: every 1kb

Geographical distribution of A genome of *Oryza*





Complex traits

- # Heading date
- # Seed dormancy
- # Eating and cooking quality
- # Spiklet number per panicle
- # Seed size
- # Plant height (Internode elongation)
- # Durable resistance to blast
- # Shattering habit
- # Resistance to ultraviolet-B
- # Phosphorous uptake

Nipponbare Kasalath

Kasalath Haberalid

Kasalath

Nipponbare

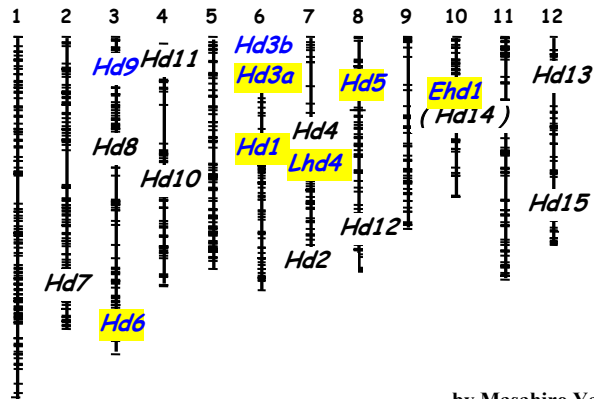
Resistant allele Susceptible allele

Kasalath Nipponbare

Kasalath Nipponbare

日本晴 NML (MIR-10)

Map-based cloning of genes controlling heading date in rice



by Masahiro Yano et al.

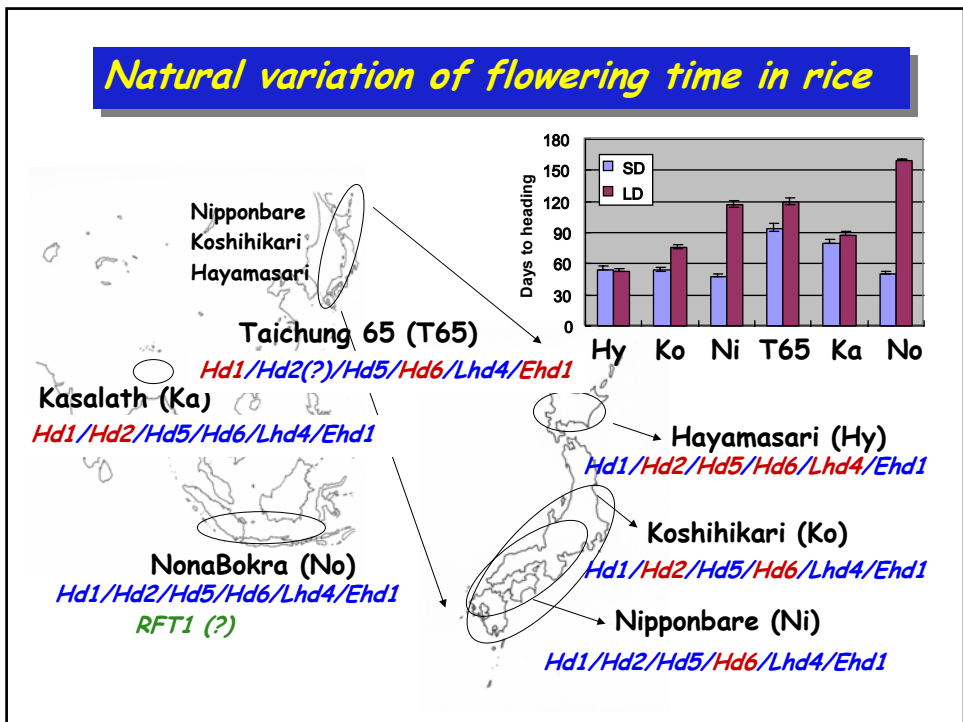
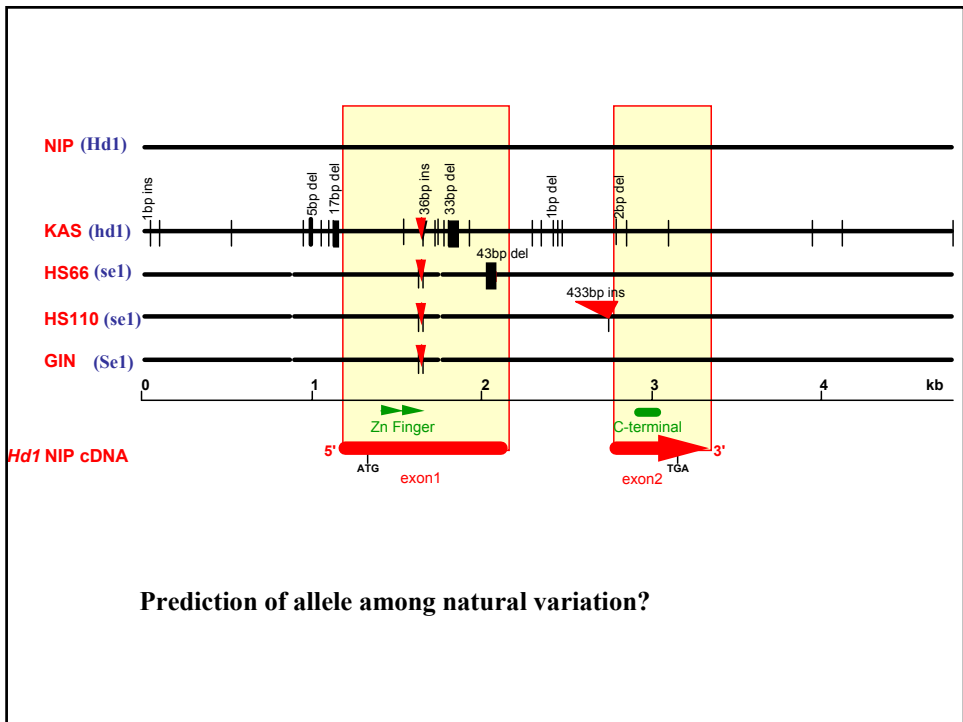
Hd1, Hd3a, Hd6, Ehd1, Hd5 and Lhd4 were cloned

Hd3b < 30 kb, Hd9 < 17 kb

Heading date QTLs identified

by Masahiro Yano et al.

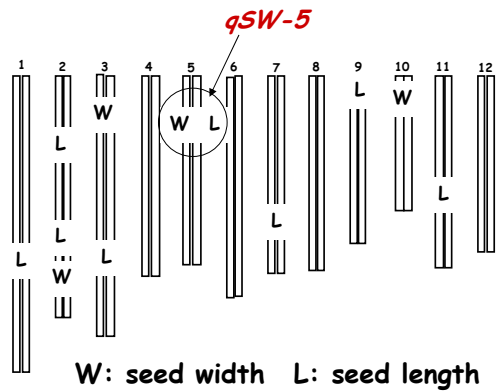
Rice	Biochemical function	Arabidopsis
Hd1 395a.a Promotion under SD Inhibition under LD	Transcription factor Zn finger domain and CCT motif	CO Promotion under LD
Hd3a 179a.a Promotion under SD	? Expression is up-regulated by Hd1	FT Promotion under LD
Hd6 333a.a Inhibition under LD	Activation factor Protein kinase CK2a	CK2a
Ehd1(Hd14) 341a.a Promotion under SD	Transcription factor B-type response regulator	No orthologue ?
Lhd4(Hd4?) 257a.a Inhibition under LD	? CCT motif	No orthologue ?
Hd5 298a.a Inhibition under LD	Transcription factor CCAAT-binding complex NF-Y subunit B (HAP3)	No orthologue ?



QTL controlling seed size



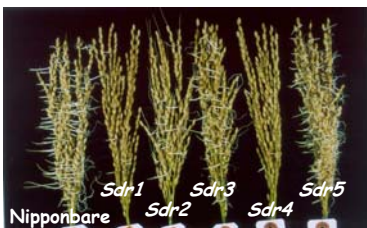
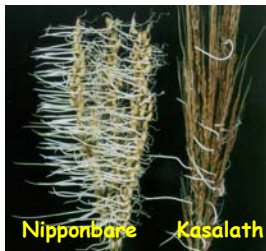
Kasalath Nipponbare



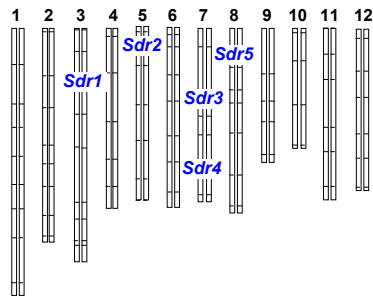
qSW-5 on chromosome 5 explained about 45% of total phenotypic variation in F2 population (Nipponbare / Kasalath)

by Shomura,A, & Yano,M.

Pre-harvest sprouting



NILs



Sdr1 : delimited to less than 2.5 kb

Sdr4 : high-resolution mapping

Sdr2 : coarse mapping

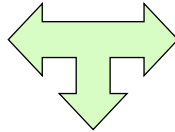
Sdr3 : coarse mapping

by Takeuchi,Y & Yano,M.

Comparison of genome of each species

What is learned and produced by this research subject?

Genome structure
Gene structure
Gene repertoire
Function of gene



History of evolution
History of domestication

What is speciation?

What factors make the identity/difference of species?

How polyploidy arose and propagated

Novel reproductive mechanism of gene transfer

Novel crops

Acknowledgement

RGP

Takashi Matsumoto
Jianzhong Wu
Nobukazu Namiki
Yuichi Katayose
Hiroyuki Kanamori
Masaki Fujisawa

NIAS

Masahiro Yano
Takeshi Izawa
Hirohiko Hirochika
Akio Miyao

IRGSP members

NIG
Takashi Gojobori
Penn.S.Univ.
Yoshihito Niimura

Funding: MAFF, BRAIN

