20. Japanese-German Symposium
Agriculture and Food Supply
Challenges and perspectives
May 8-9, 2015

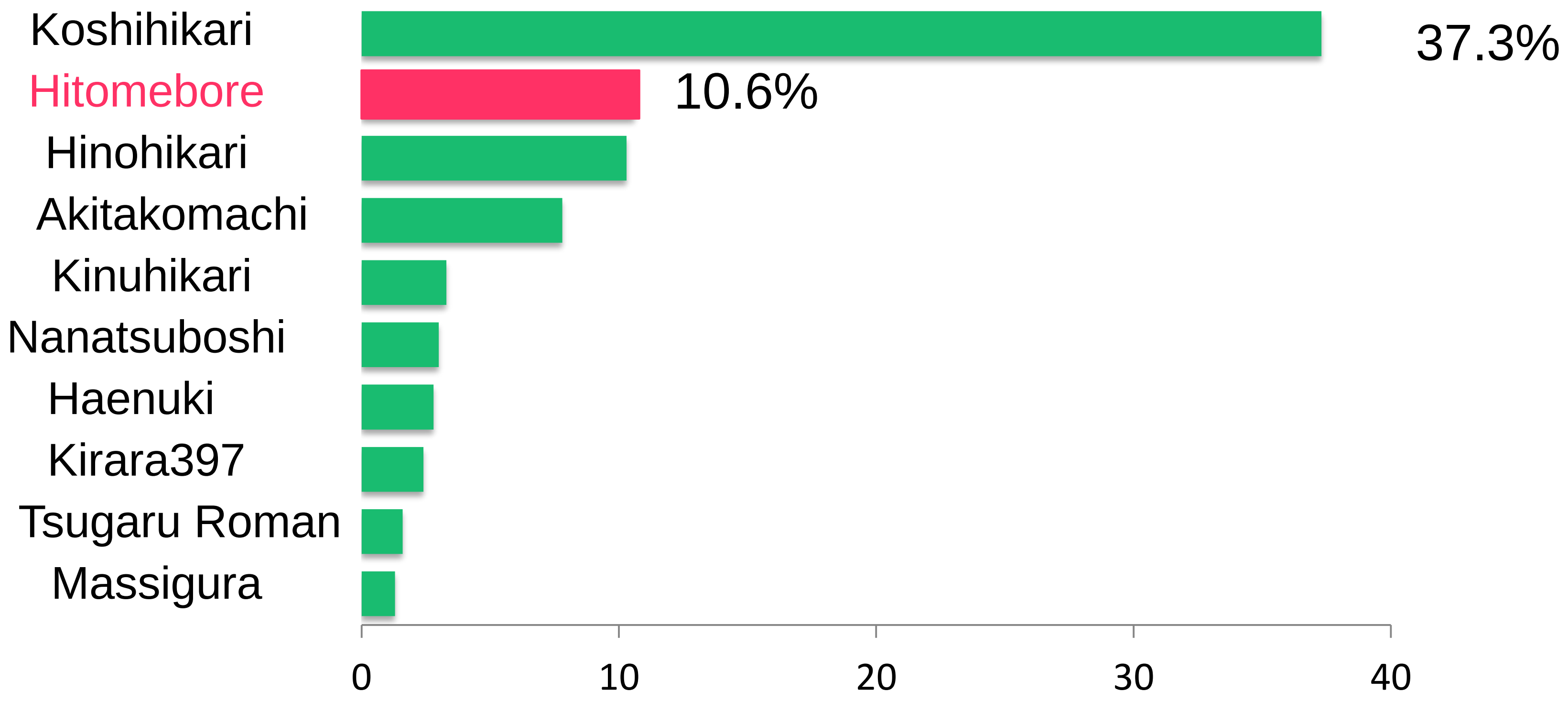
Genome Sequencing and Modern Breeding

Ryohei Terauchi

Iwate Biotechnology Research Center
Kitakami, Iwate, Japan

Iwate Region = Agriculture

Cultivation area of rice cultivars (Japan)



（MAFF）

Hitomebore: Major elite cultivar in Miyagi, Iwate, Fukushima

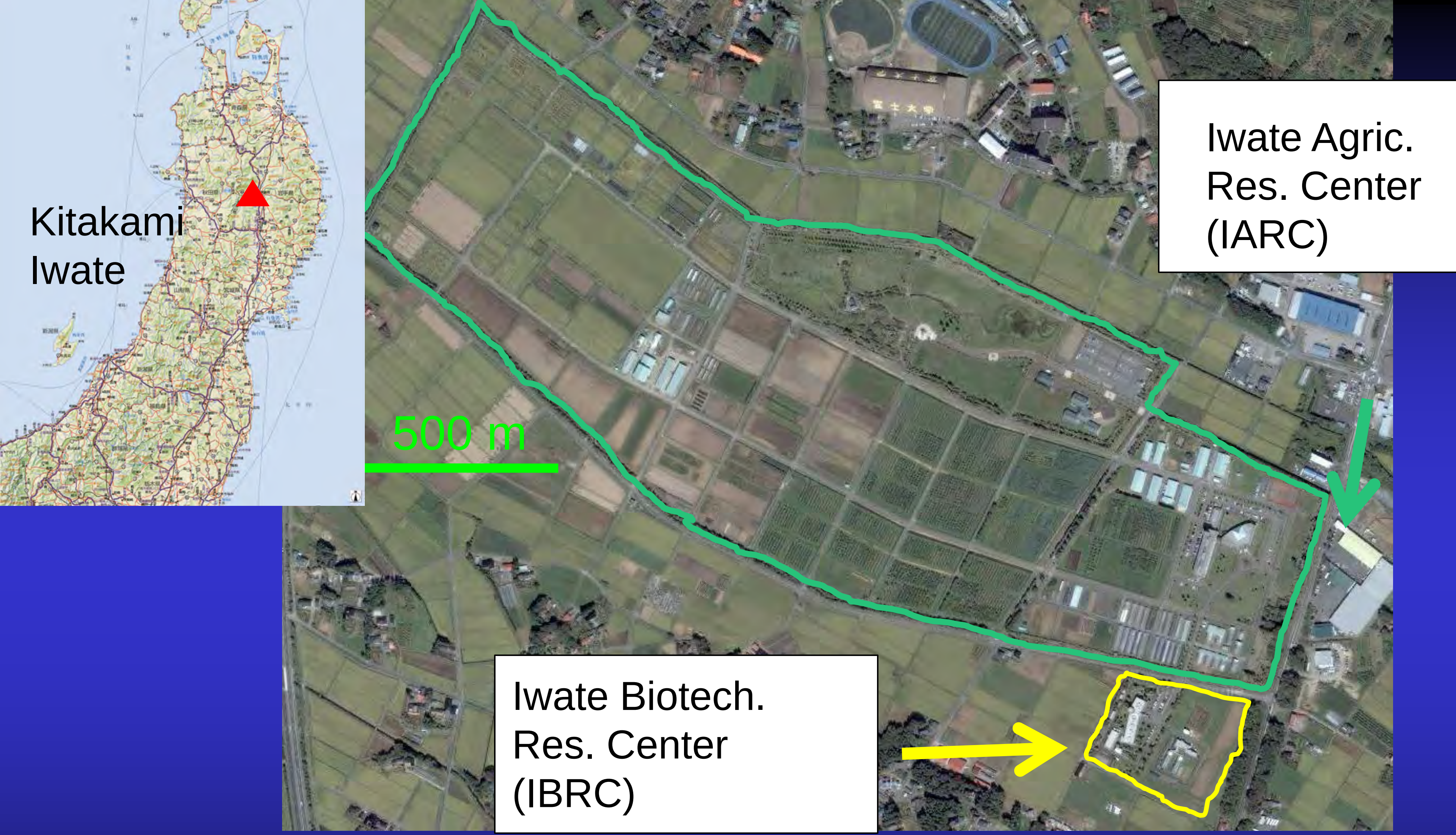
Rice cultivar “Hitomebore”

Major elite cultivar of Tohoku region

Improvements needed:

- Blast disease resistance
- Cold tolerance
- Eating quality
- Seedling vigor
- Yield

→ Deliver safer and
more competitive cultivars to farmers



Kitakami
Iwate

500 m

Iwate Agric.
Res. Center
(IARC)

Iwate Biotech.
Res. Center
(IBRC)

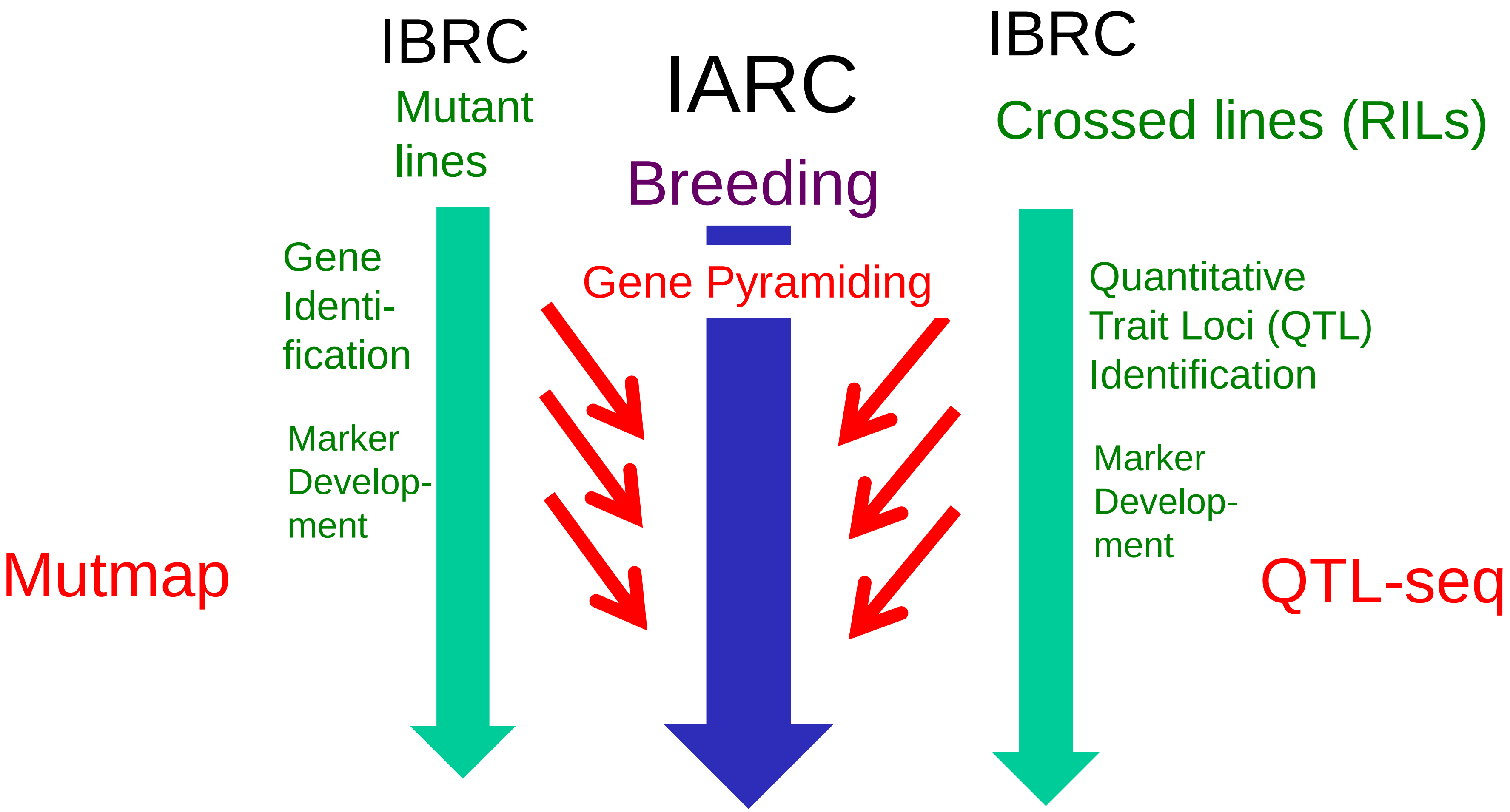
Non-profit research organization
Funded by Iwate Local Government
40 researchers
Major mandates: rice
gentian

Rice Breeding in Iwate

Elite cultivar
“Hitomebore”

Target Traits: Blast resistance, Cold tolerance
Eating quality, Seedling vigor, Yield

Non transgenic



IBRC
Mutant lines

Gene Identification

Marker Development

Mutmap

IARC
Breeding

Gene Pyramiding

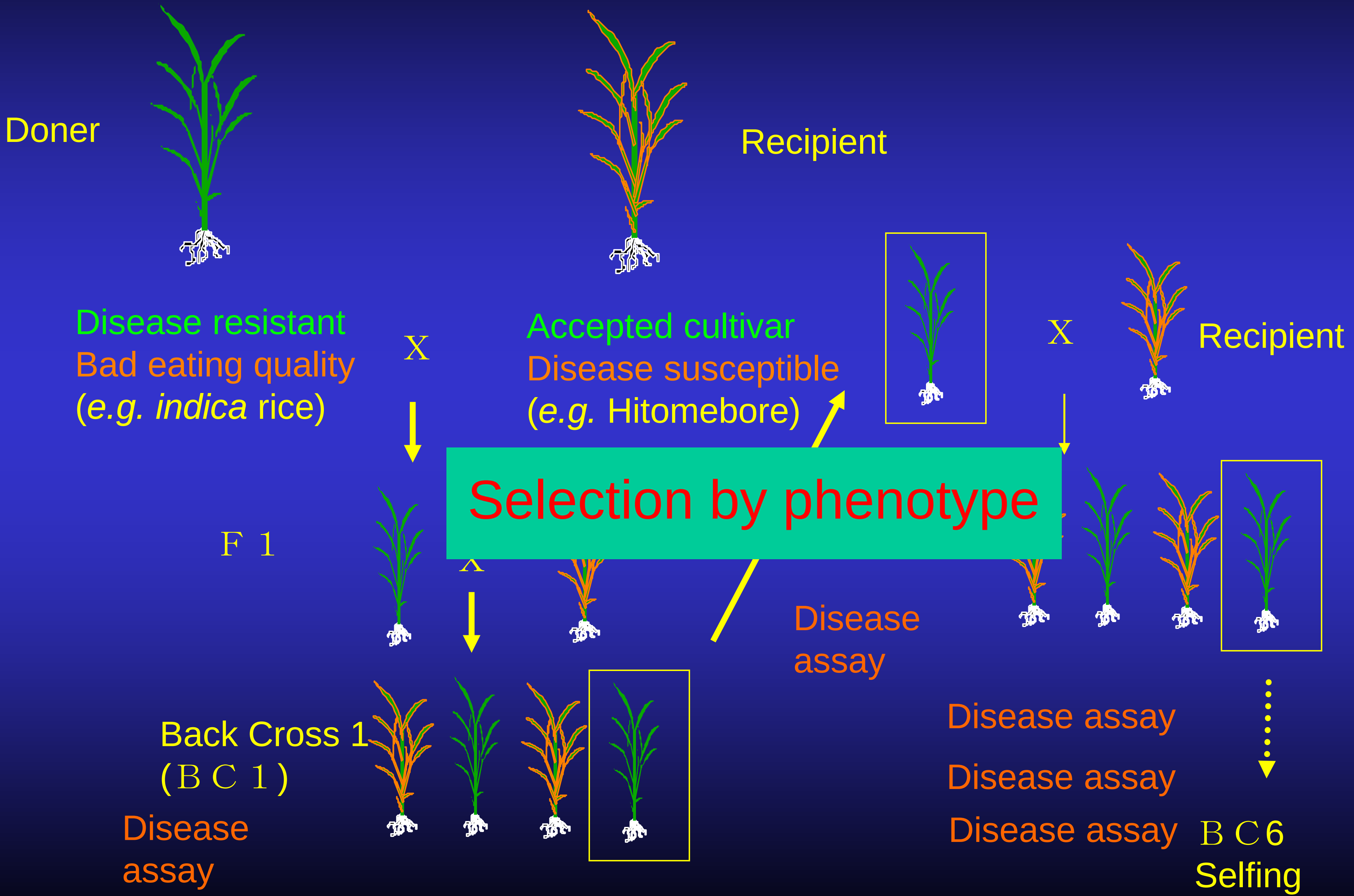
IBRC
Crossed lines (RILs)

Quantitative Trait Loci (QTL) Identification

Marker Development

QTL-seq

Conventional breeding: time consuming and labor intensive



Doner

Recipient

Disease resistant
Bad eating quality
(*e.g. indica* rice)

X

Accepted cultivar
Disease susceptible
(*e.g.* Hitomebore)

X

Recipient

Selection by phenotype

F 1

X

Disease
assay

Back Cross 1
(B C 1)

Disease
assay

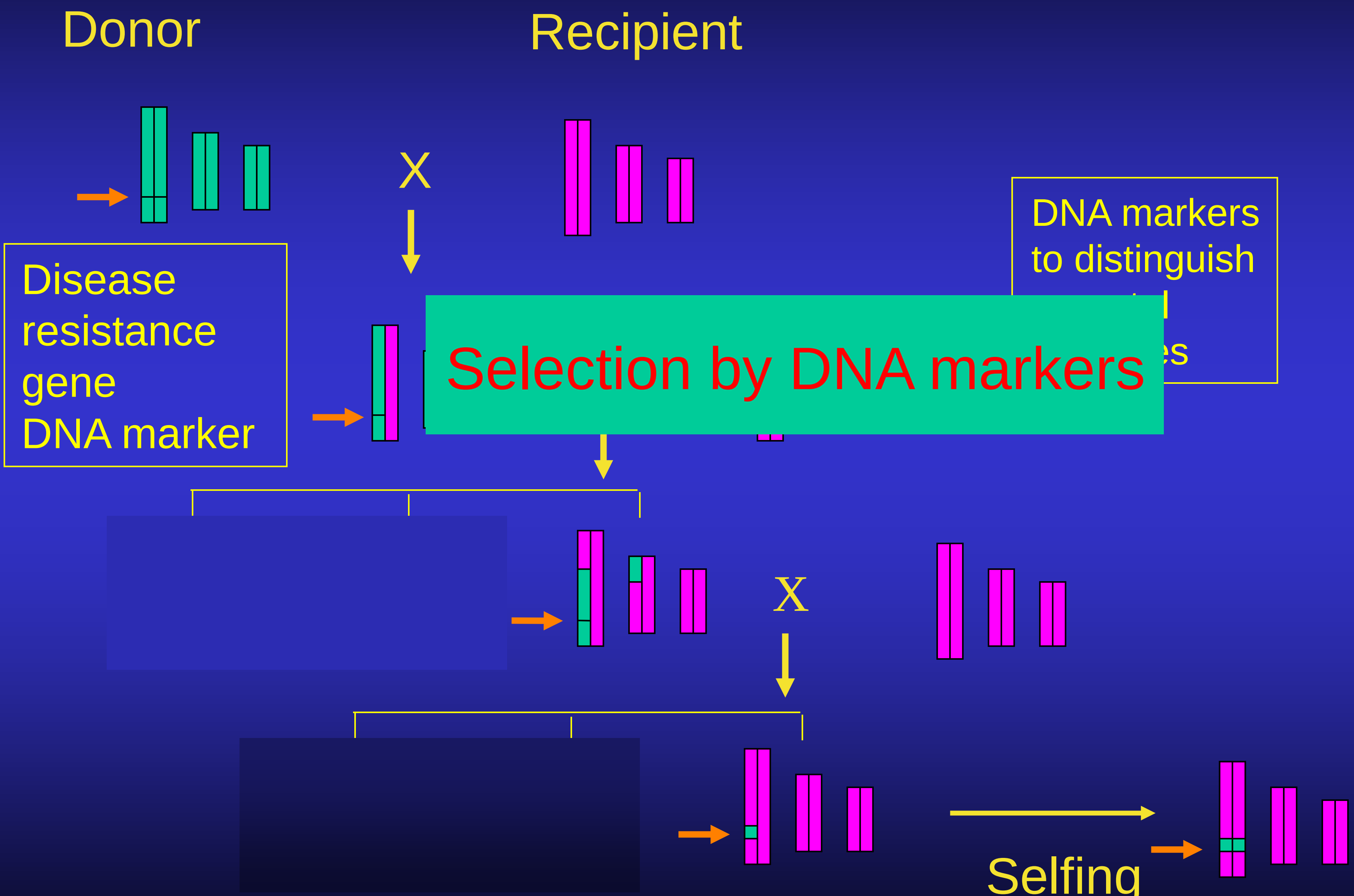
Disease assay

Disease assay

Disease assay

B C 6
Selfing

Modern breeding: DNA marker assisted selection



Donor

Recipient

X

Disease resistance gene DNA marker

DNA markers to distinguish

es

Selection by DNA markers

X

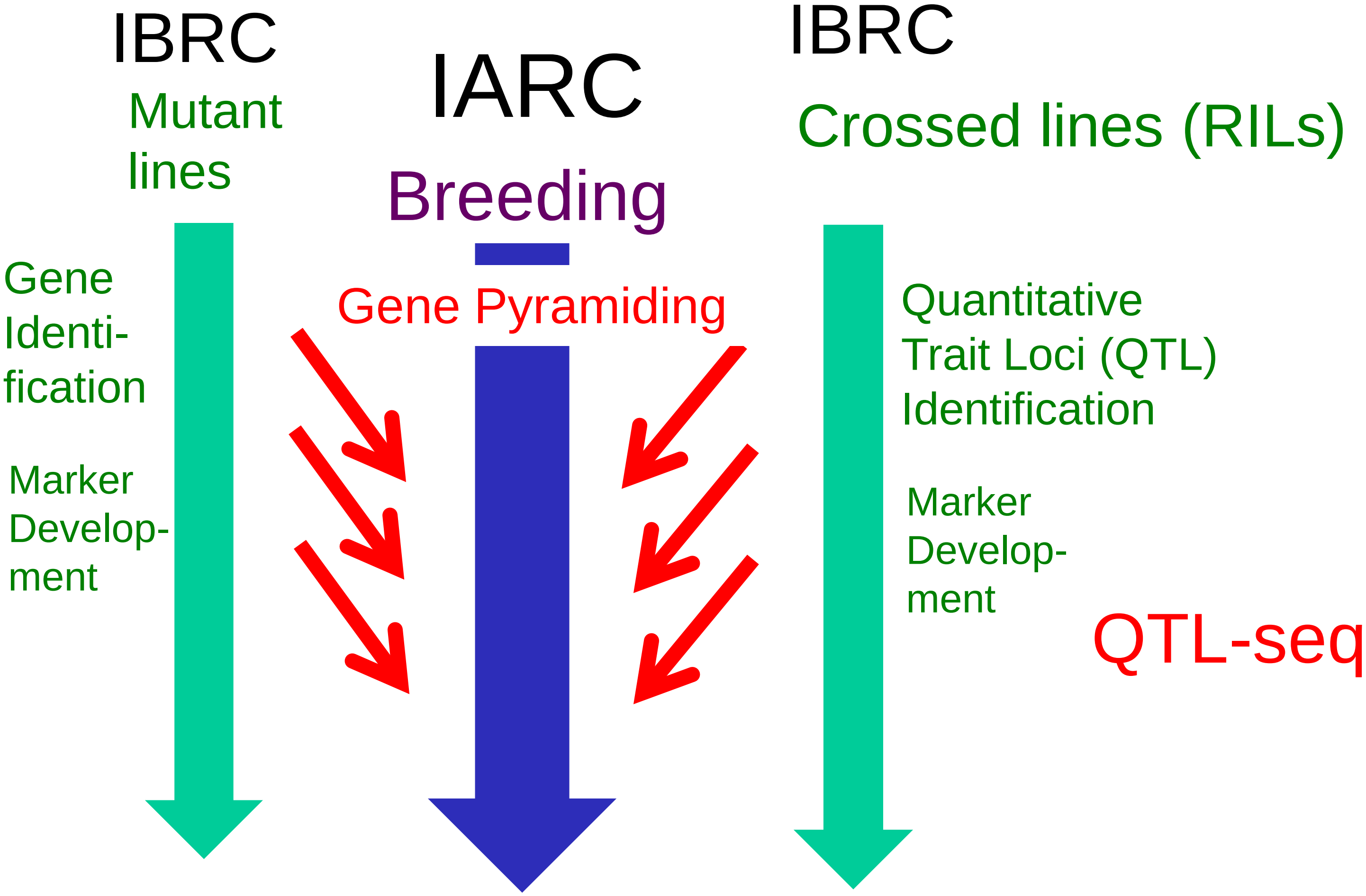
Selfing

Rice Breeding in Iwate

Elite cultivar
“Hitomebore”

Target Traits: Blast resistance, Cold tolerance
Eating quality, Seedling vigor, Yield

Non transgenic



IBRC
Mutant lines

IARC
Breeding

IBRC
Crossed lines (RILs)

Gene Identification

Marker Development

Mutmap

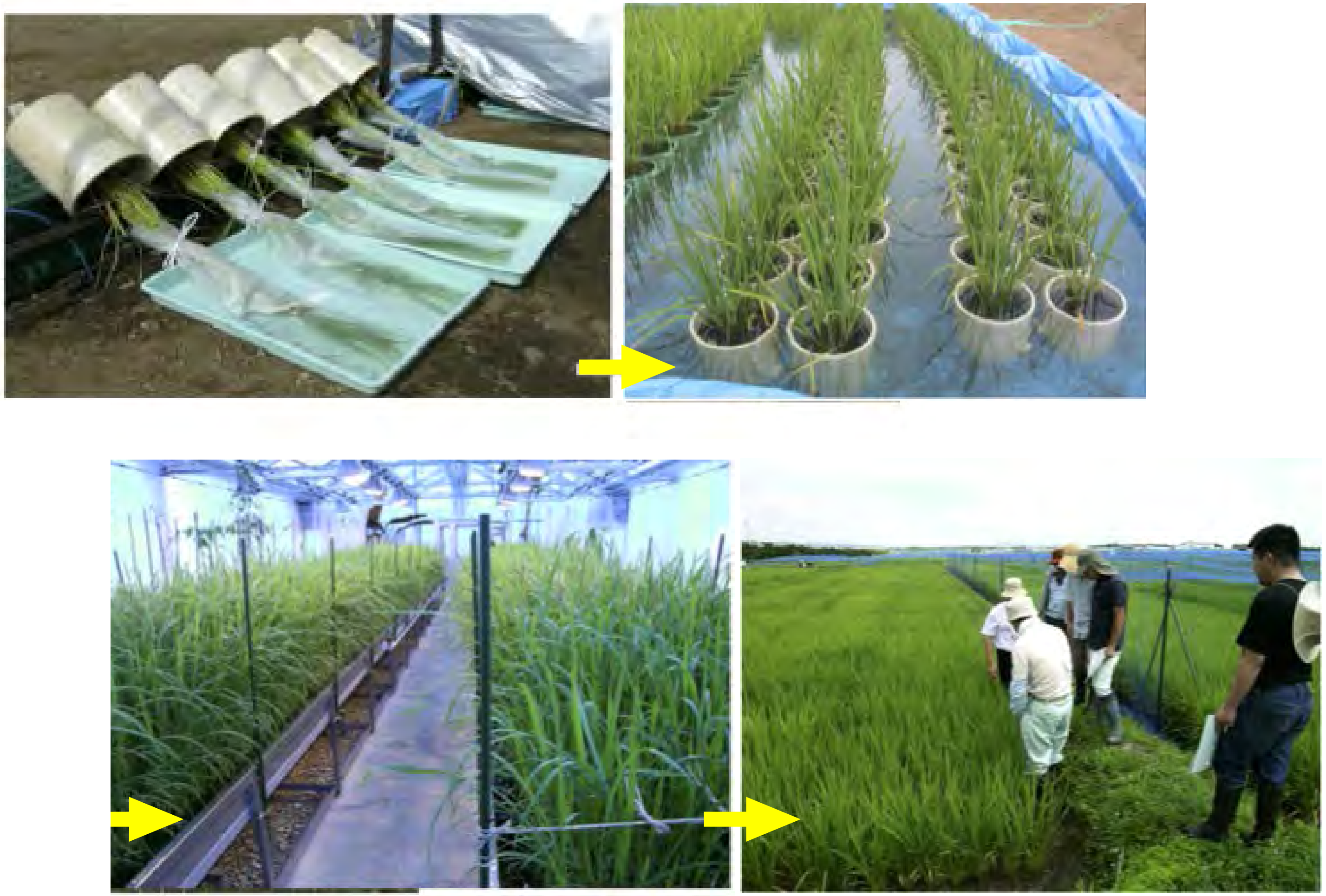
Gene Pyramiding

Quantitative Trait Loci (QTL) Identification

Marker Development

QTL-seq

EMS mutagenesis of Hitomebore



12,000 M3-M5 lines

~1,500 Single *N*ucleotide *P*olymorphisms (SNPs / line)

Crossed Lines (Recombinant Inbred Lines: RILs) 2008-

Hitomebore x

Total: 3,172 lines
（F8+F6）

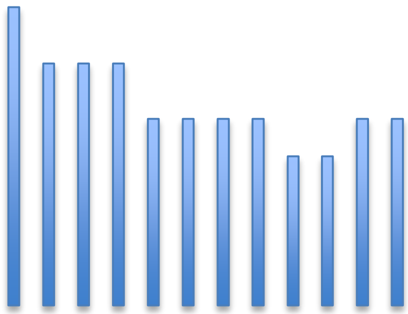
Accession	Origin	F2 seeds
WRC2	India	450
WRC4	Nepal	200
WRC17	China	345
WRC21	Nepal	310
WRC31	Bangladesh	1630
WRC32	Bangladesh	530
WRC39	Nepal	240
WRC41	Srilanka	2670
WRC47	Brazil	715
WRC50	USA	965
WRC51	Japan	340
WRC55	Bangladesh	840
WRC98	China	304
W0106	O. rufipogon	710
W0120	O. rufipogon	332
W2003	O. rufipogon	250
NERICA		1280

Trait evaluation of mutants and crossed lines (RILs)

Data management by bar-codes

Rice genome

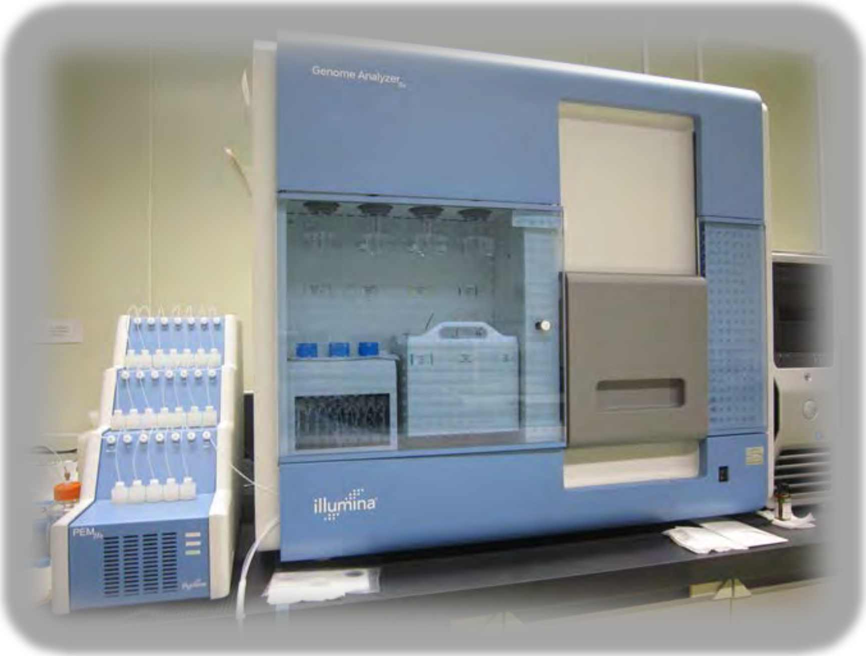
12 chromosomes



- Accurate reference genome sequence (cv. Nipponbare) published in 2005
- 389 Mb = 389 million bases (nucleotides)
- 38,000 protein coding genes

→ Gene function largely unknown

IBRC next generation sequencing (NGS) platform (2010-)



Illumina Genome Analyzer IIx (GAIIx)

“Most widely adopted NGS system”

50 Gb
A single run takes
~ 7 days

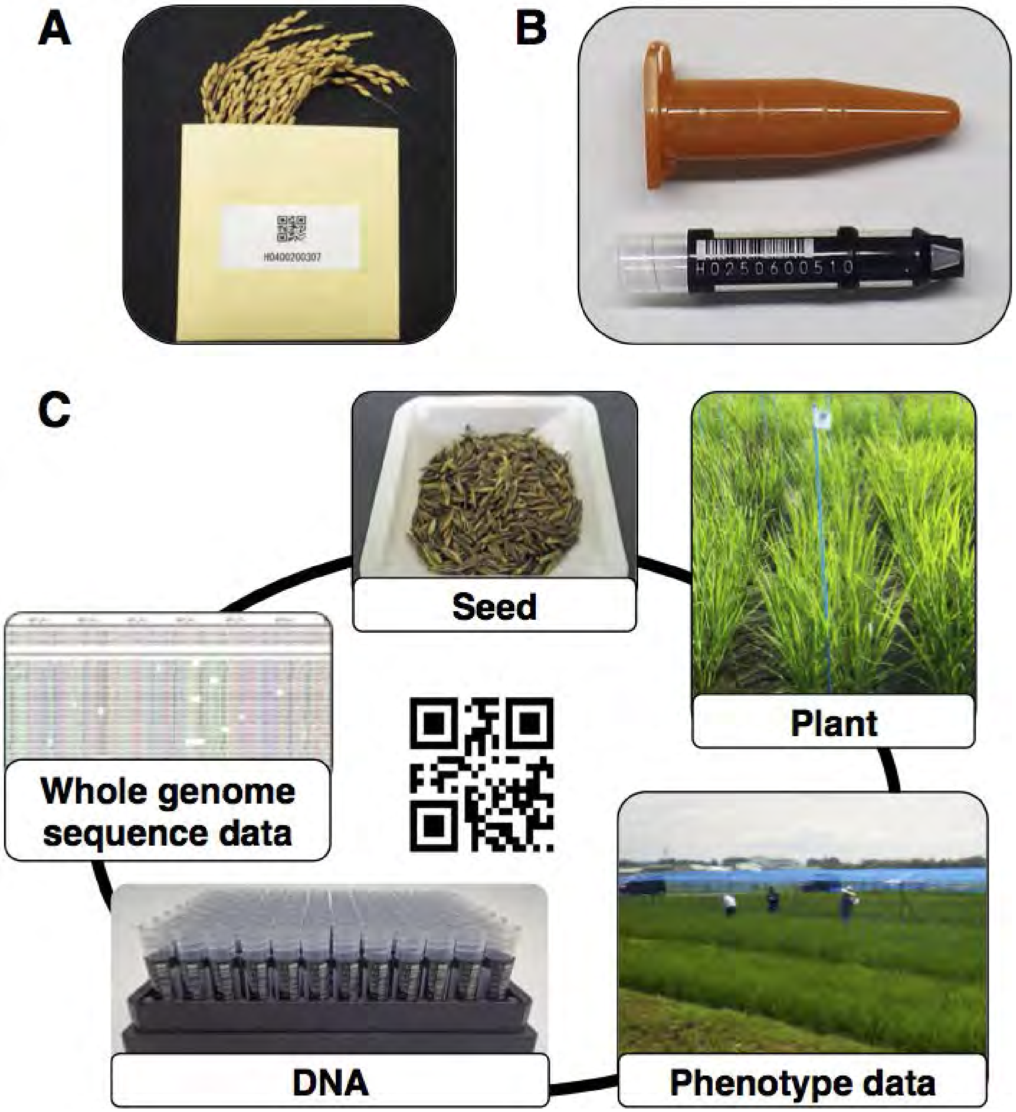
Disk
22 Tera byte

Memory
300 Gb

Giga bit network

Bioinformatics
4 Bioinformaticians

Genome wide association (GWAS) study by WGS



MutMap method

ARTICLES

nature
biotechnology

Received 28 July 2011; accepted 14 December 2011; published online 22 January 2012; doi:10.1038/nbt.2095

Genome sequencing reveals agronomically important loci in rice using MutMap

Akira Abe[1,2,7], Shunichi Kosugi[3,7], Kentaro Yoshida[3], Satoshi Natsume[3], Hiroki Takagi[2,3], Hiroyuki Kanzaki[3], Hideo Matsumura[3,4], Kakoto Yoshida[3], Chikako Mitsuoka[3], Muluneh Tamiru[3], Hideki Innan[5], Liliana Cano[6], Sophien Kamoun[6] & Ryohei Terauchi[3]

The majority of agronomic traits are controlled by multiple genes of these genes difficult. Here we introduce MutMap, a method b segregating population of plants that show a useful phenotype. I line and then selfed, allowing unequivocal segregation in second This approach is particularly amenable to crop species because mutant F_2 progeny that are required. We applied MutMap to sev unique genomic positions most probable to harbor mutations ca relevant trait. These results show that MutMap can accelerate th

The world population is predicted to reach 9 billion within the next 40 years, requiring a 70–100% increase in food production relative to current levels[1]. It is a major challenge to ensure sustainable food production without further expanding farmland and damaging the environment, in the midst of adverse conditions such as rapid climatic

A
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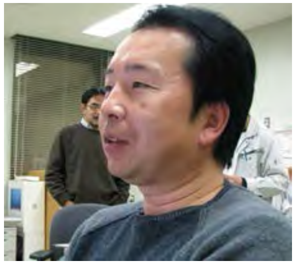
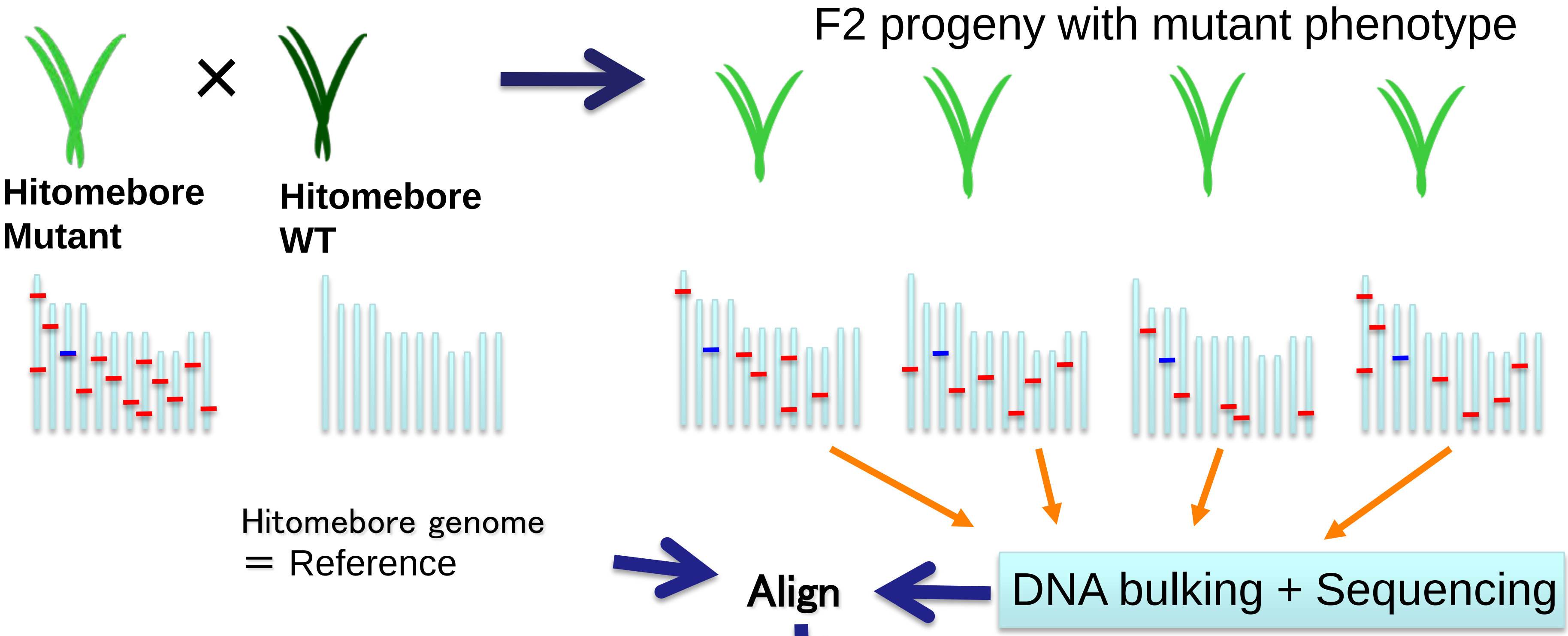


Shunichi
Kosugi

MutMap method

Abe *et al.* Nat. Biotech. 2012



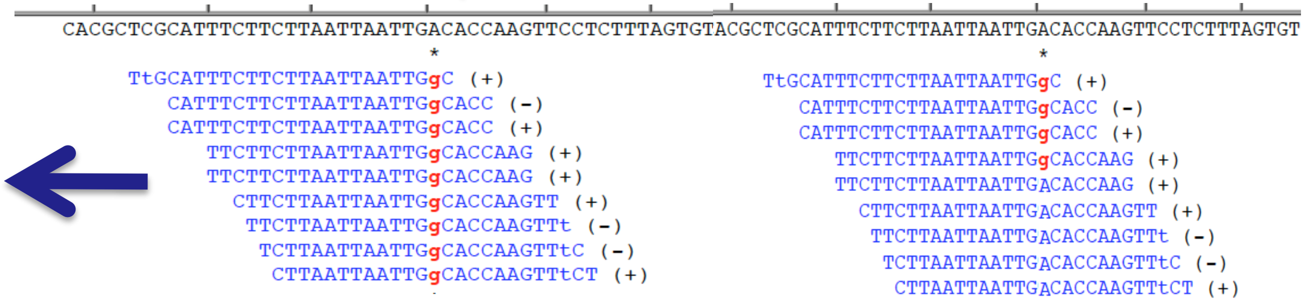
F2 progeny with mutant phenotype

Hitomebore Mutant × **Hitomebore WT**

Hitomebore genome
＝ Reference

Align

DNA bulking + Sequencing



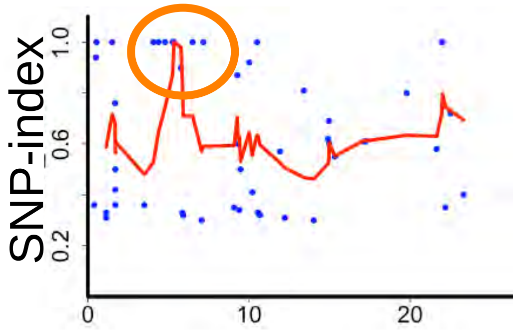
```
CACGCTCGCATTTCTTCTTAATTAATTGACACCAAGTTCCTCTTTAGTGTACGCTCGCATTTCTTCTTAATTAATTGACACCAAGTTCCTCTTTAGTGT
                                            *                                                    *
```

```
TtGCATTTCTTCTTAATTAATTGgC (+)
    CATTTCTTCTTAATTAATTGgCACC (-)
    CATTTCTTCTTAATTAATTGgCACC (+)
        TTCTTCTTAATTAATTGgCACCAAG (+)
        TTCTTCTTAATTAATTGgCACCAAG (+)
           CTTCTTAATTAATTGgCACCAAGTT (+)
             TTCTTAATTAATTGgCACCAAGTTt (-)
              TCTTAATTAATTGgCACCAAGTTtC (-)
                CTTAATTAATTGgCACCAAGTTtCT (+)
```

```
TtGCATTTCTTCTTAATTAATTGgC (+)
   CATTTCTTCTTAATTAATTGgCACC (-)
   CATTTCTTCTTAATTAATTGgCACC (+)
      TTCTTCTTAATTAATTGgCACCAAG (+)
      TTCTTCTTAATTAATTGACACCAAG (+)
        CTTCTTAATTAATTGACACCAAGTT (+)
         TTCTTAATTAATTGACACCAAGTTt (-)
          TCTTAATTAATTGACACCAAGTTtC (-)
           CTTAATTAATTGACACCAAGTTtCT (+)
```

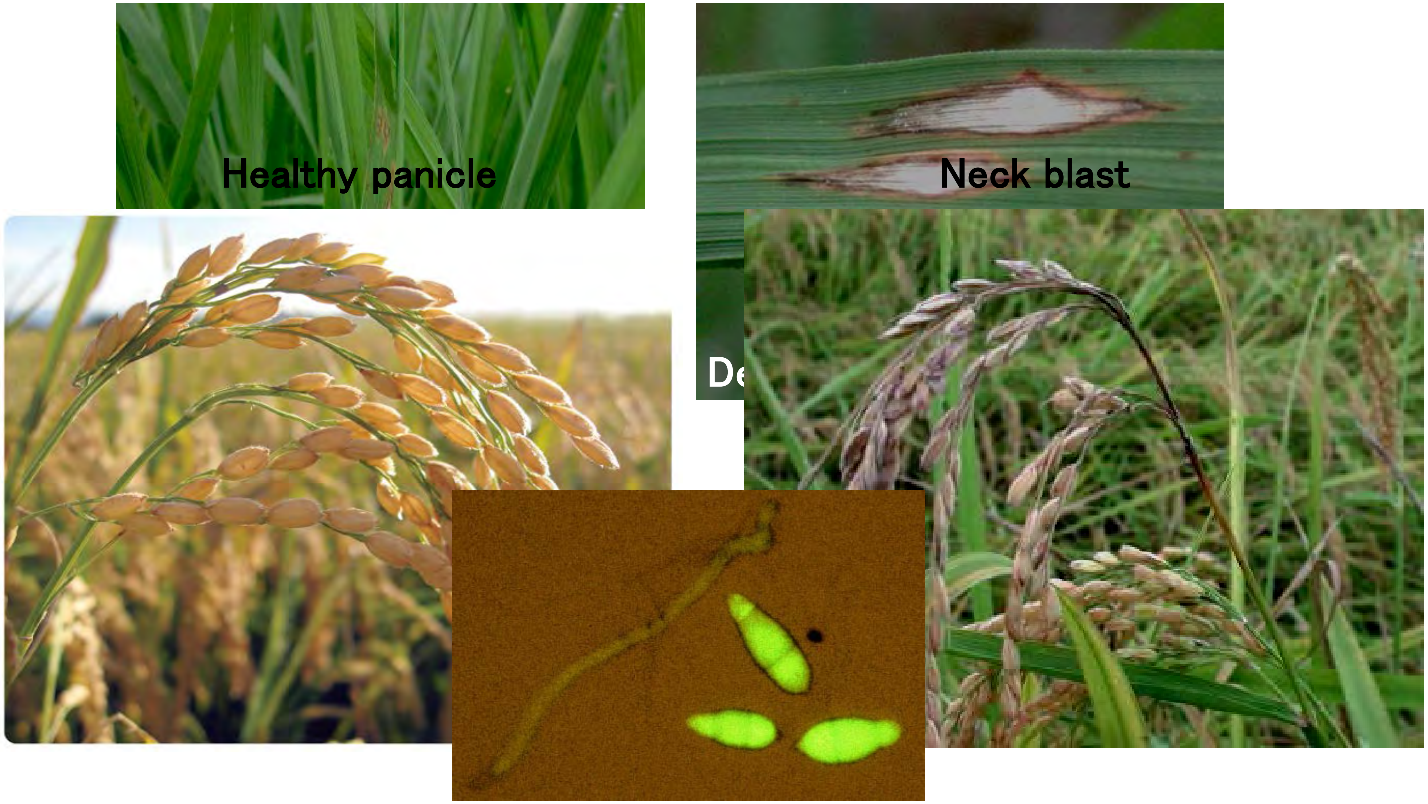
SNP-index = 9/9= 1

SNP-index=4/9=0.44
~ 0.5



Plot SNP-index on chromosomal location

Rice blast disease caused by *Magnaporthe oryzae*



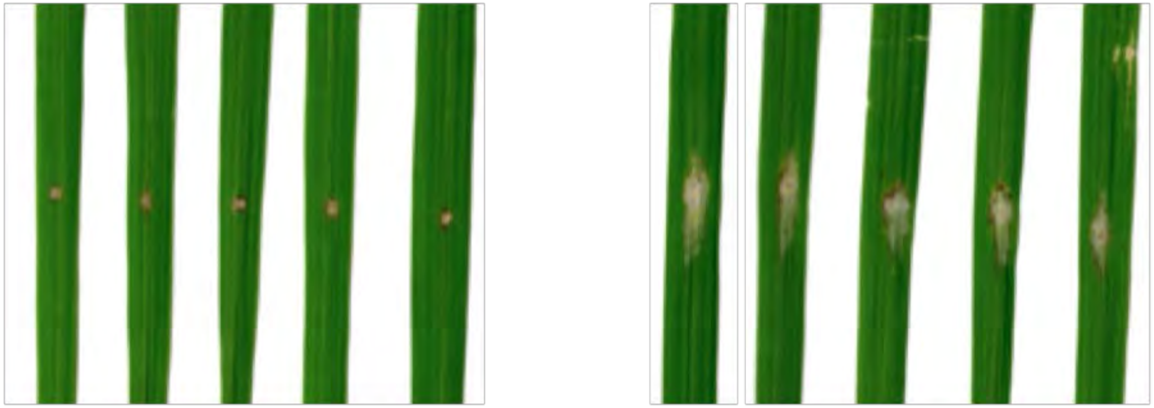
MutMap identifies a blast resistance gene *Pii*

Mutant : Hit 5948

Hitomebore (*Pii*) X Hit 5948

↓

F_2



M. oryzae
with *AVR-Pii*
(ligand)

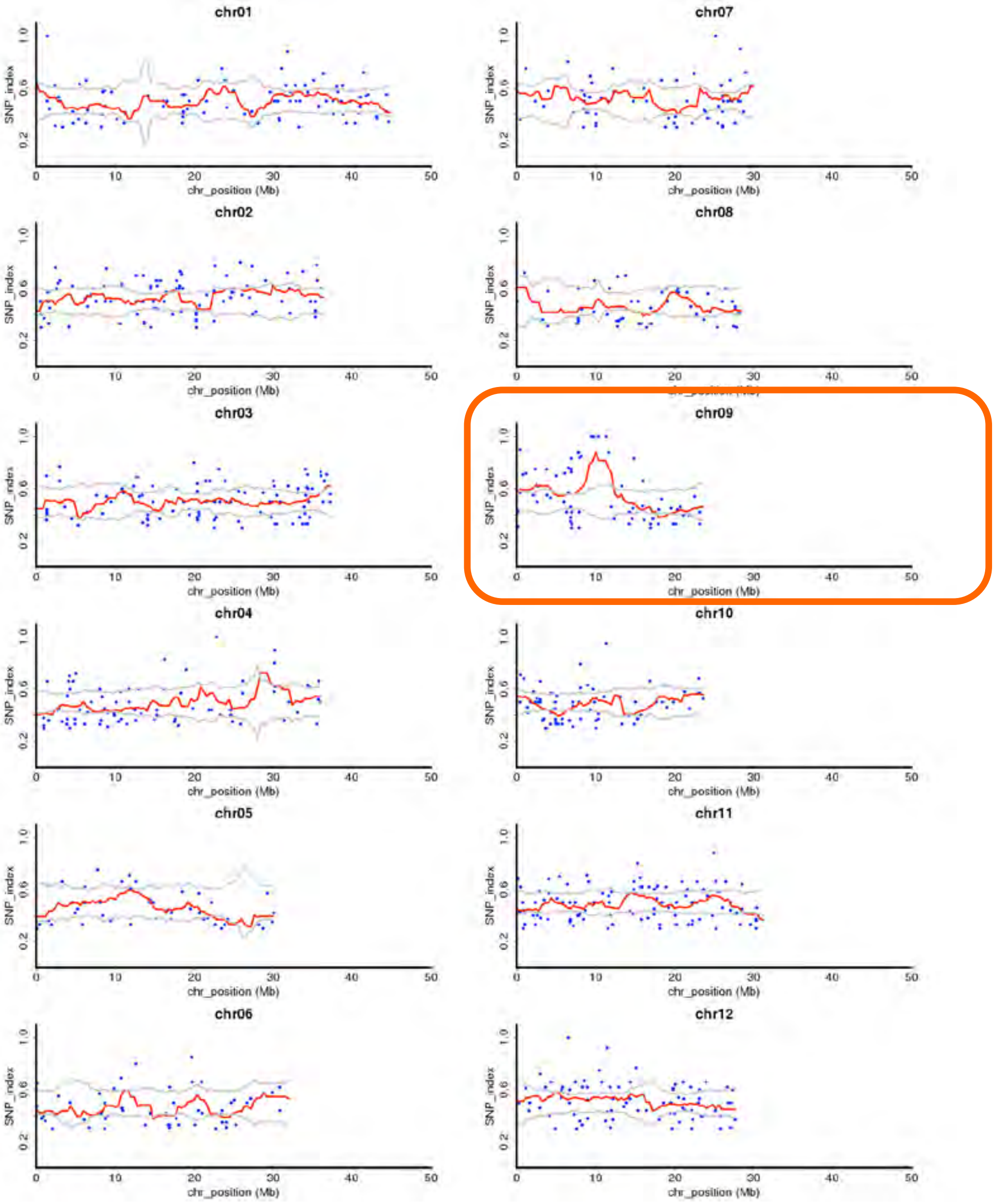
WT

Mutant progeny

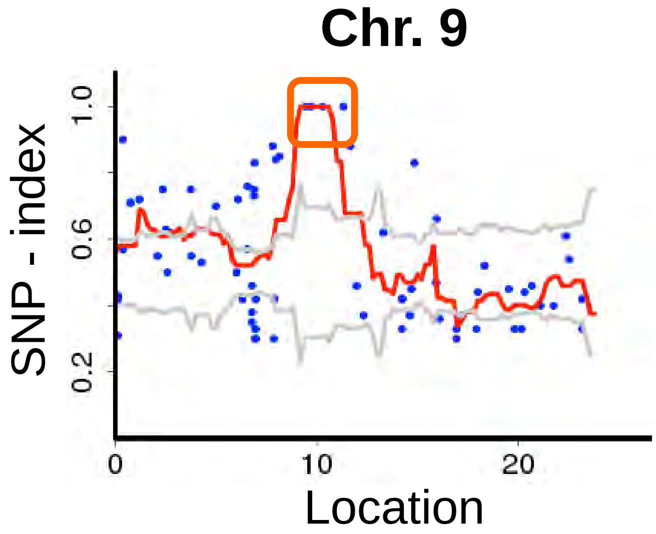
61 : 17 → MutMap

MutMap identifies a blast resistance gene *Pii*

MutMap result



MutMap identifies a blast resistance gene *Pii*



Gene: Os09t0327600-01

Position 10290916

nt change: G to A

aa change: W to Stop (TGG to TGA)

WT

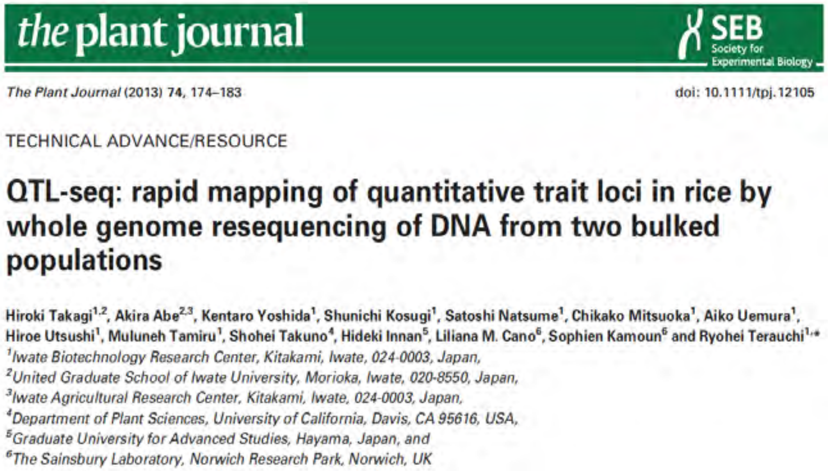
MVGAEMLVAAAVSQVARKINGIVGVAQGEVKLCCNFSDDLEGIK
DTLVYLETLLKNAENNSFGSDRANLRHWLGQIKSLAYDIEDIVDG
YYSSKEQFDGGSYAQKGSLFCSLSNPMLLKGSMVYKMKSKREM
LQQRQQLPNQYHFLSHINSAVNFEEKQTTSYRNTDIAIVGRDADL
DNLMDILMENSAEELSIISIVGPVGFGKTSLAQFVFNNTGTEVFSF
RI W VHVSMGNINLEKIGRDIVSQTTEKIEGNMQLQSIKNAVQ
RVLNKYSCLIILDSLWGKDEEVNELKQMLLTGRHTESKIIVTTHSN
KVAKLISTVPLYKLAALSEDDCLKIFSQRAMTGPGDPLFREYGEEI
VRRCEGTPLVANFLGSVVNAQRQRREIWQAAKDEEMWKIEEDY
PQDKISPLFPSFKIIYYNMPHELRLCFVYCSIFPKGTVIEKKKLIQQ
WIALDMIEC

Mutant

MVGAEMLVAAAVSQVARKINGIVGVAQGEVKLCCNFSDDLEGIK
DTLVYLETLLKNAENNSFGSDRANLRHWLGQIKSLAYDIEDIVDG
YYSSKEQFDGGSYAQKGSLFCSLSNPMLLKGSMVYKMKSKREM
LQQRQQLPNQYHFLSHINSAVNFEEKQTTSYRNTDIAIVGRDADL
DNLMDILMENSAEELSIISIVGPVGFGKTSLAQFVFNNTGTEVFSF
RI * VHVSMGNINLEKIGRDIVSQTTEKIEGNMQLQSIKNAVQRV
LNKYSCLIILDSLWGKDEEVNELKQMLLTGRHTESKIIVTTHSNKV
AKLISTVPLYKLAALSEDDCLKIFSQRAMTGPGDPLFREYGEEIVR
RCEGTPLVANFLGSVVNAQRQRREIWQAAKDEEMWKIEEDYPQ
DKISPLFPSFKIIYYNMPHELRLCFVYCSIFPKGTVIEKKKLIQQWIA
LDMIEC

Takagi *et al.* New Phytol. 2013

QTL-seq method applied to RILs (Crossed lines)



the plant journal SEB Society for Experimental Biology

The Plant Journal (2013) 74, 174–183 doi: 10.1111/tpj.12105

TECHNICAL ADVANCE/RESOURCE

QTL-seq: rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations

Hiroki Takagi[1,2], Akira Abe[2,3], Kentaro Yoshida[1], Shunichi Kosugi[1], Satoshi Natsume[1], Chikako Mitsuoka[1], Aiko Uemura[1], Hiroe Utsushi[1], Muluneh Tamiru[1], Shohei Takuno[4], Hideki Innan[5], Liliana M. Cano[6], Sophien Kamoun[6] and Ryohei Terauchi[1,*]

[1]*Iwate Biotechnology Research Center, Kitakami, Iwate, 024-0003, Japan,*
[2]*United Graduate School of Iwate University, Morioka, Iwate, 020-8550, Japan,*
[3]*Iwate Agricultural Research Center, Kitakami, Iwate, 024-0003, Japan,*
[4]*Department of Plant Sciences, University of California, Davis, CA 95616, USA,*
[5]*Graduate University for Advanced Studies, Hayama, Japan, and*
[6]*The Sainsbury Laboratory, Norwich Research Park, Norwich, UK*

Takagi, H. *et al.* 2013
Plant J. 74:174

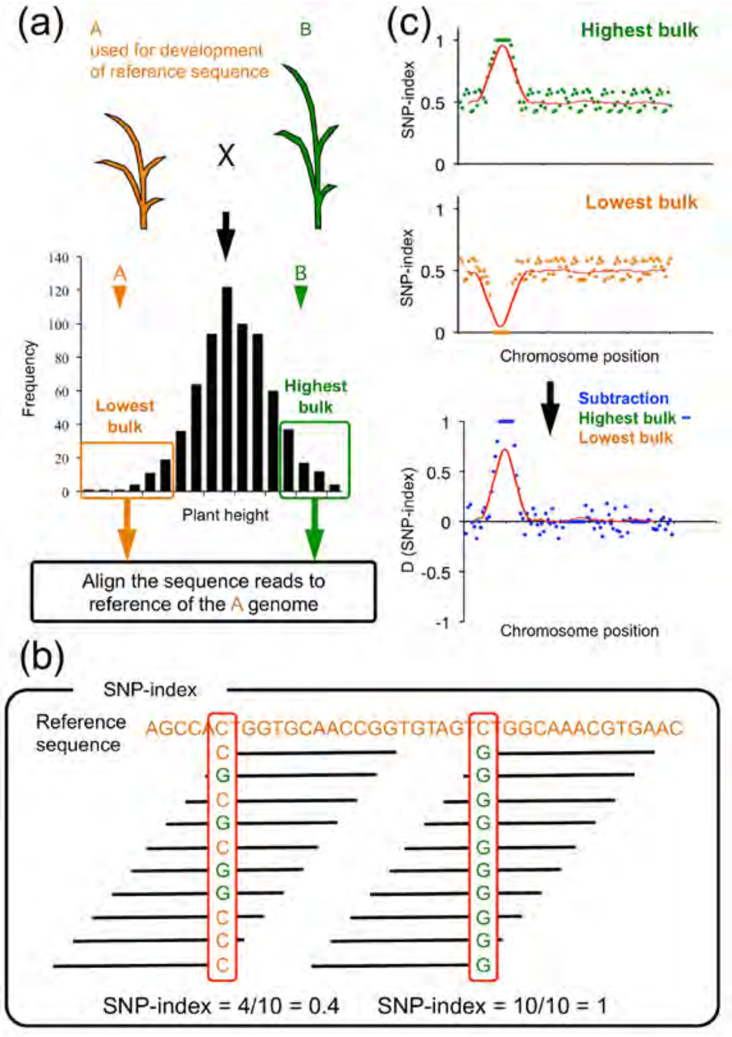
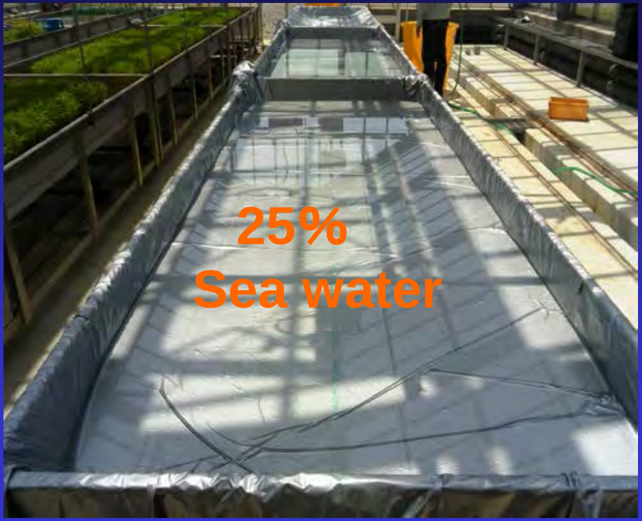


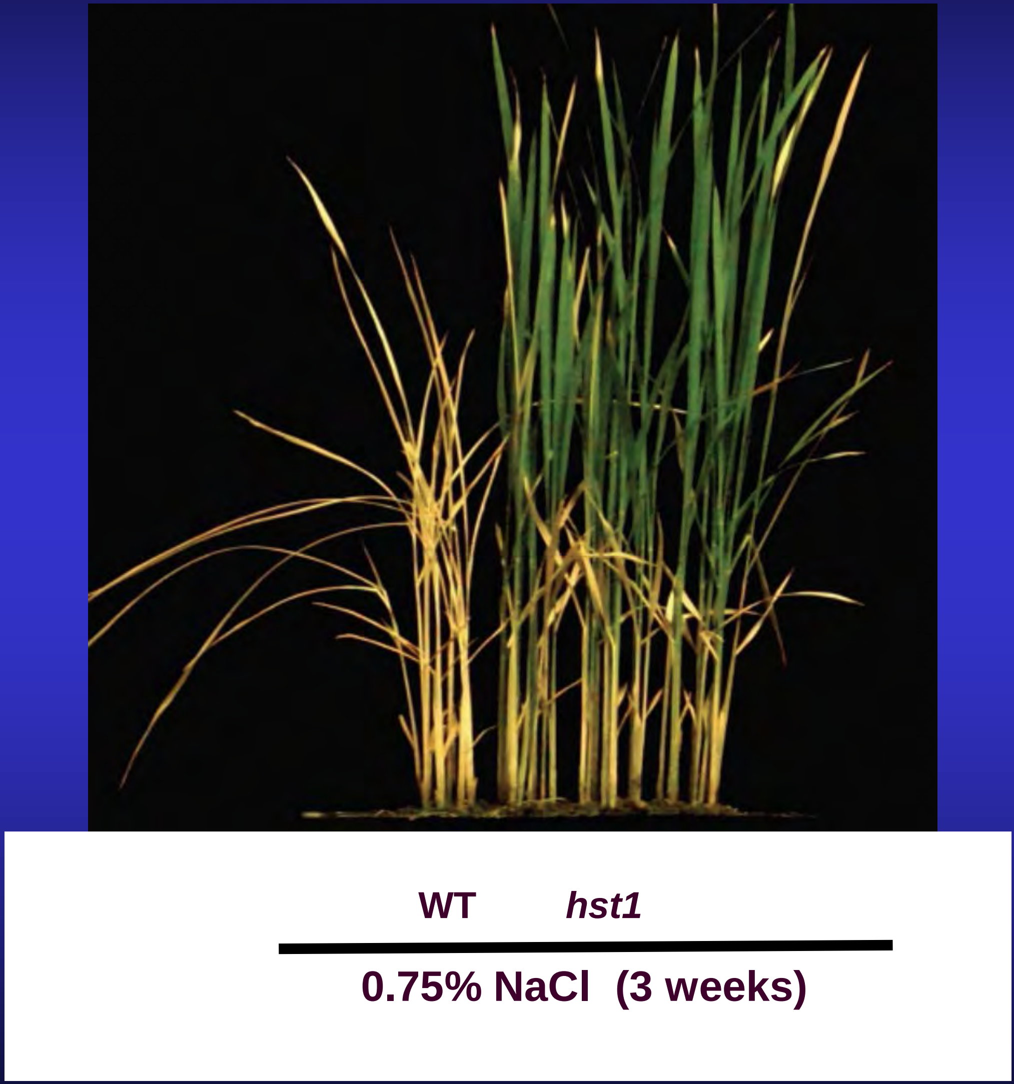
Figure 1.

Hiroki Takagi

Tsunami-flooded paddy field of Iwate Coast (April 11, 2011)

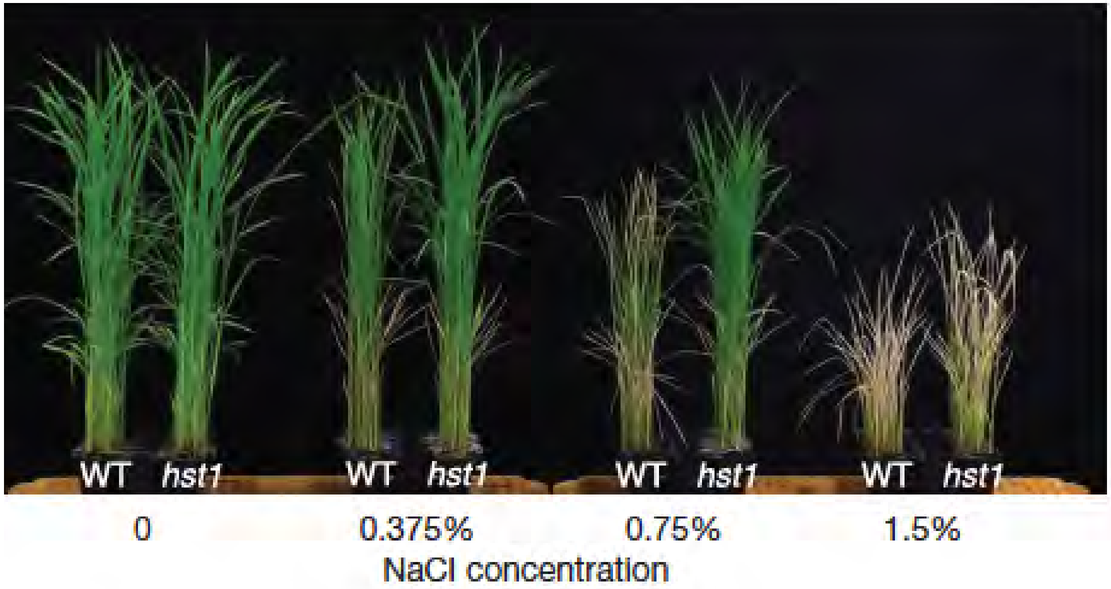
Isolation of a Hitomebore salt-tolerant (*hst1*) mutant



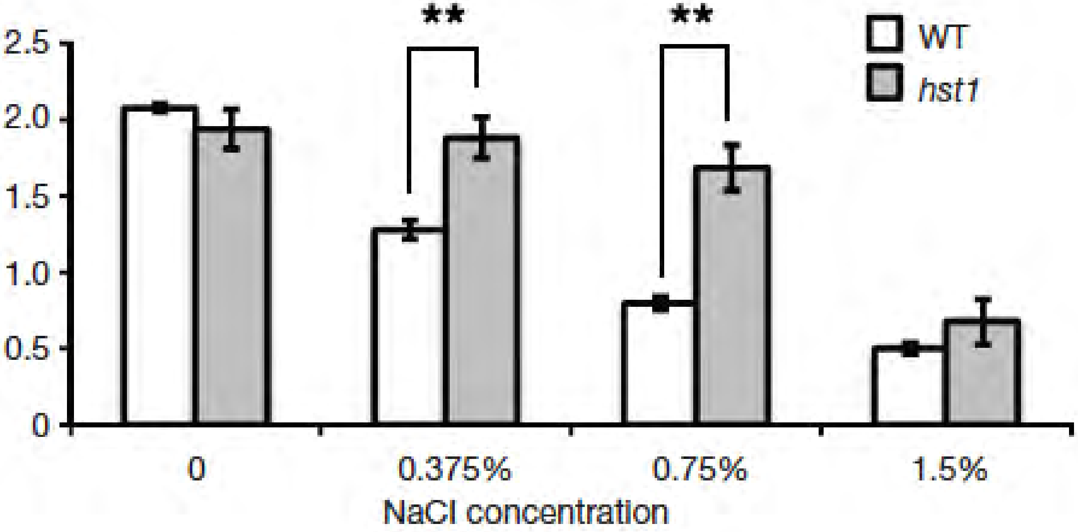


0.75% NaCl (3 weeks)

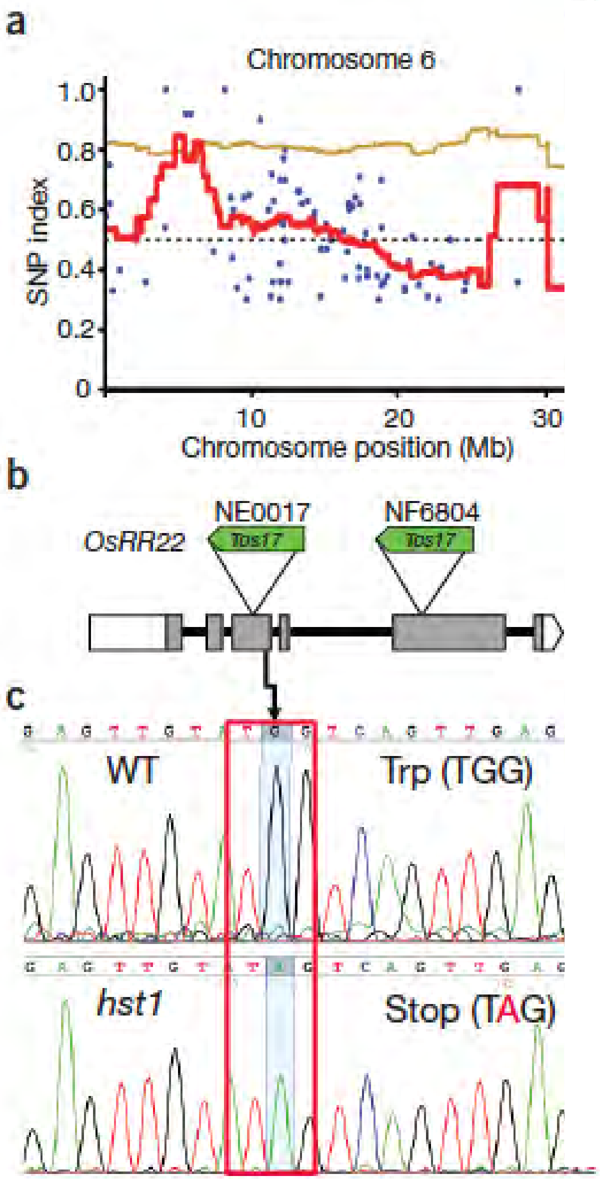
Identification of *hst1* mutant with salinity tolerance



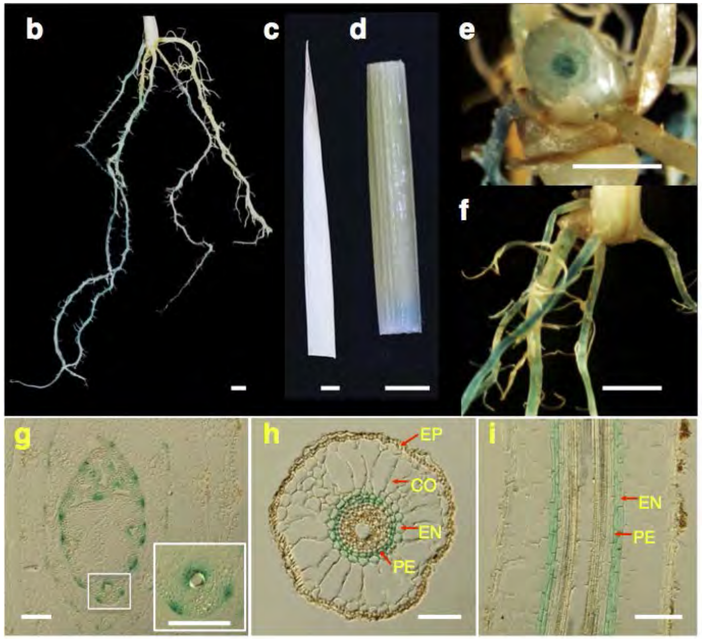
Fresh Weight (g)



MutMap identifies *OsRR22* gene responsible for salt tolerance

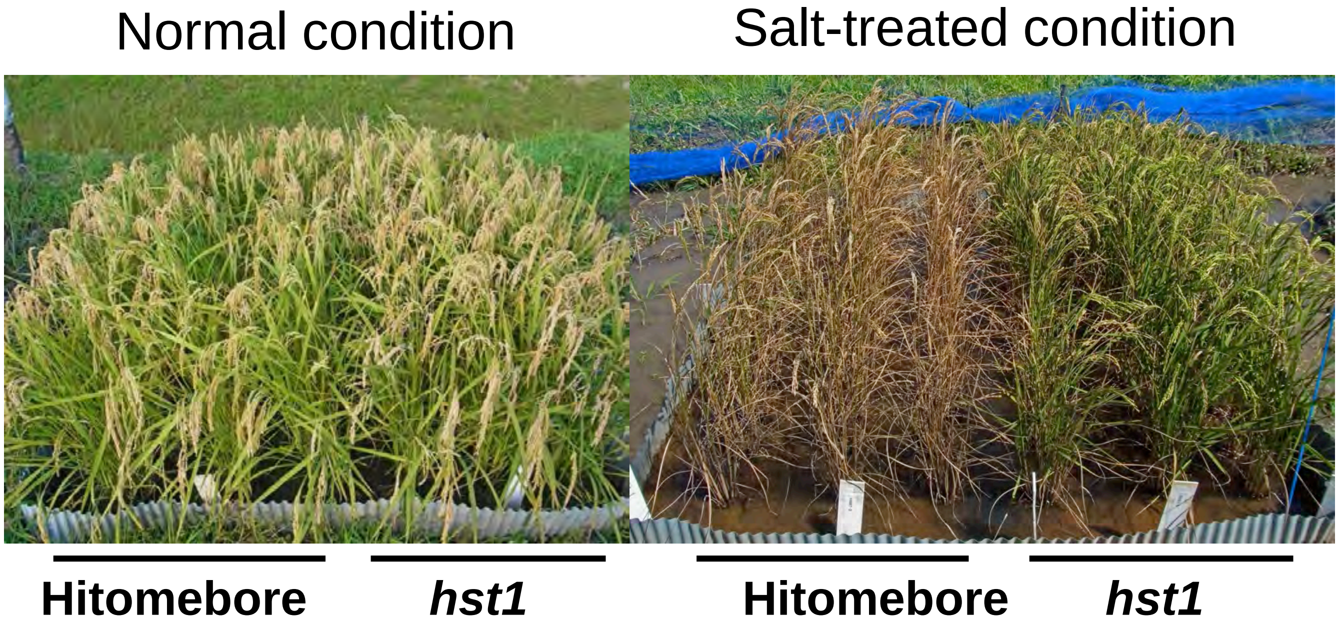


OsRR22:
hisitidine kinase
signaling pathway
gene



Expression of *OsRR22*
endodermis/pericycle of root/stem

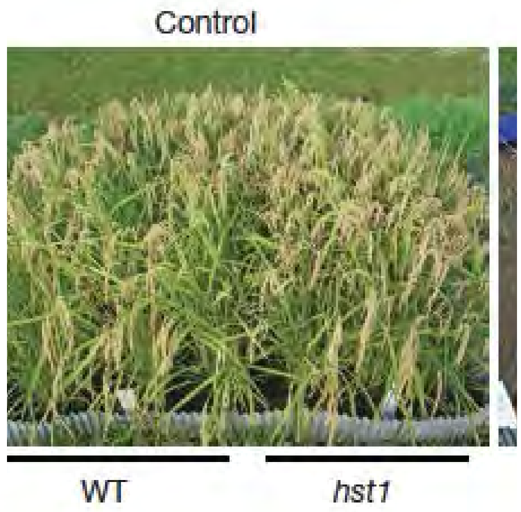
hst1 shows salt tolerance in field



Normal condition

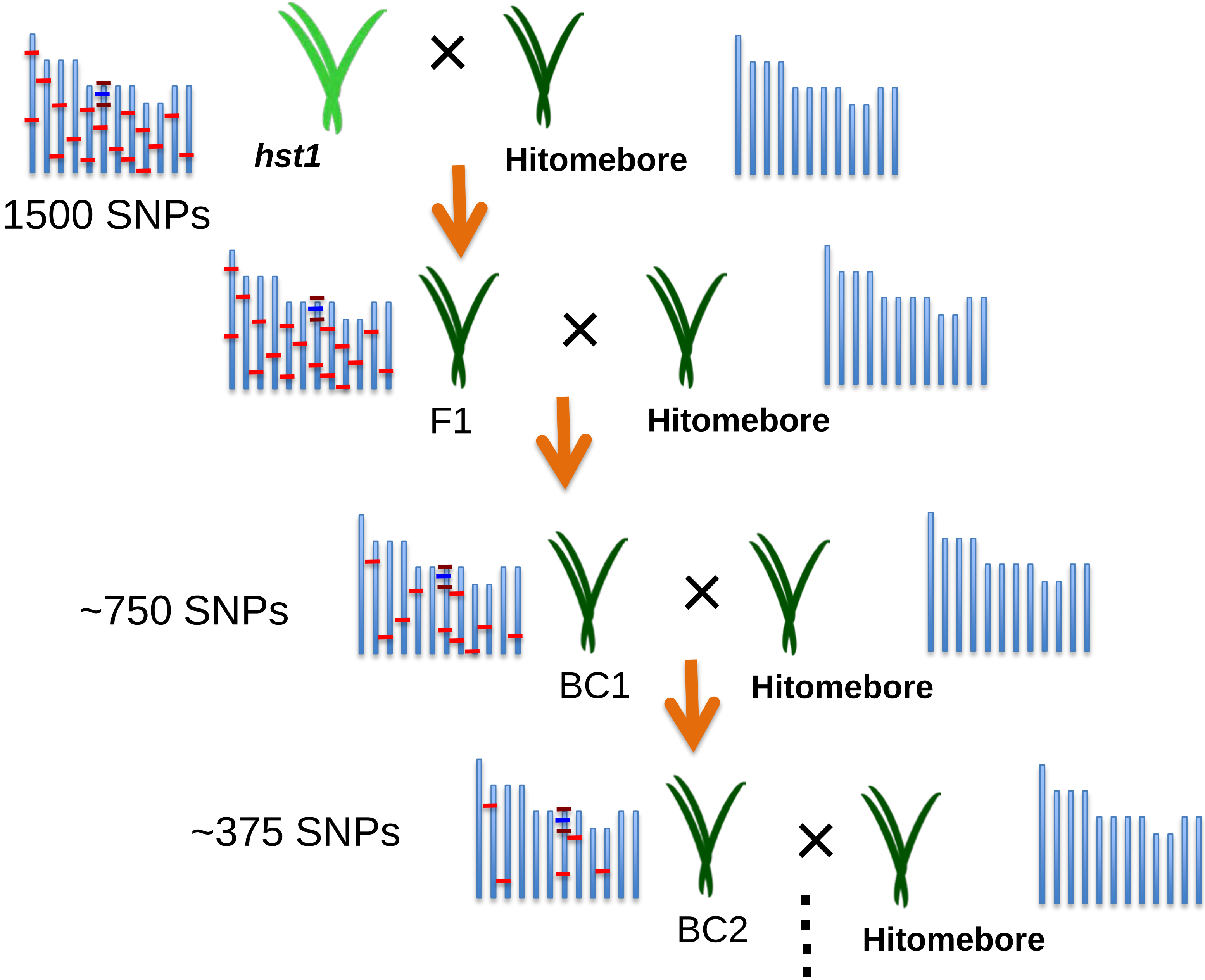
Salt-treated condition

Yield



Control

Backcross of *hst1* to Hitomebore to reduce unnecessary mutations

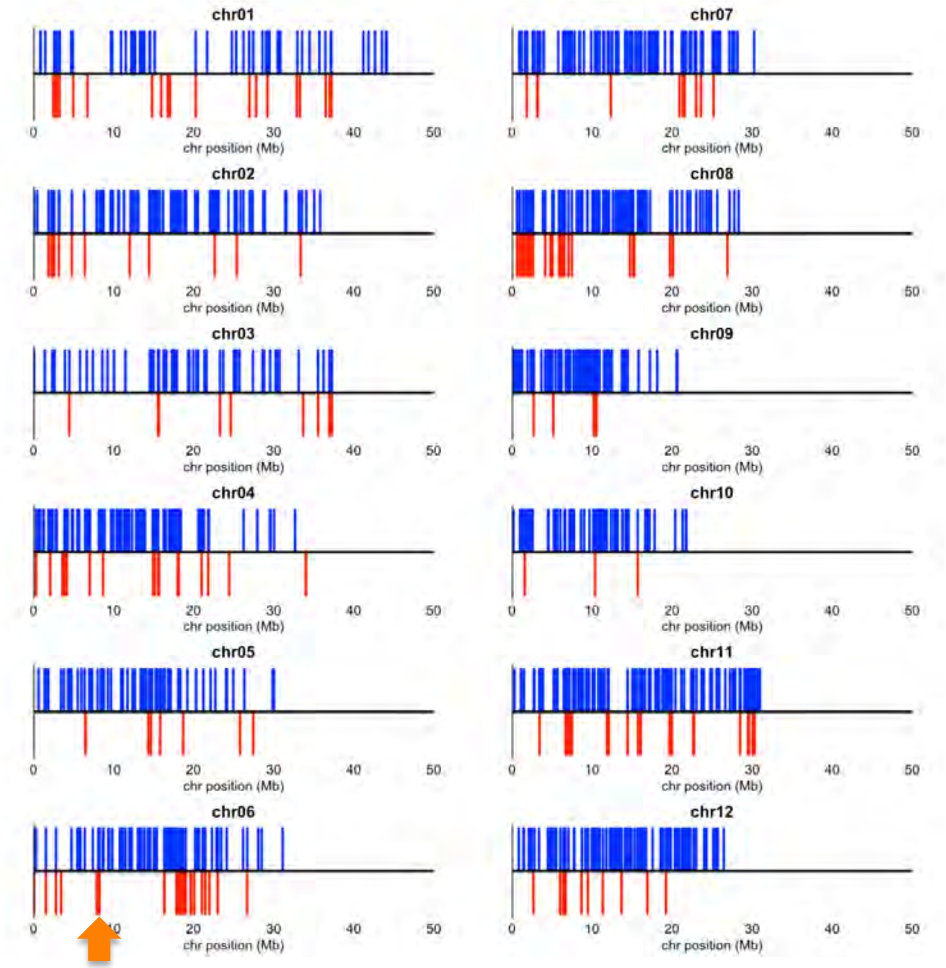


A variety “Kaijin” differs from Hitomebore only by 201 SNPs

“Kaijin” in Japanese = Neptune

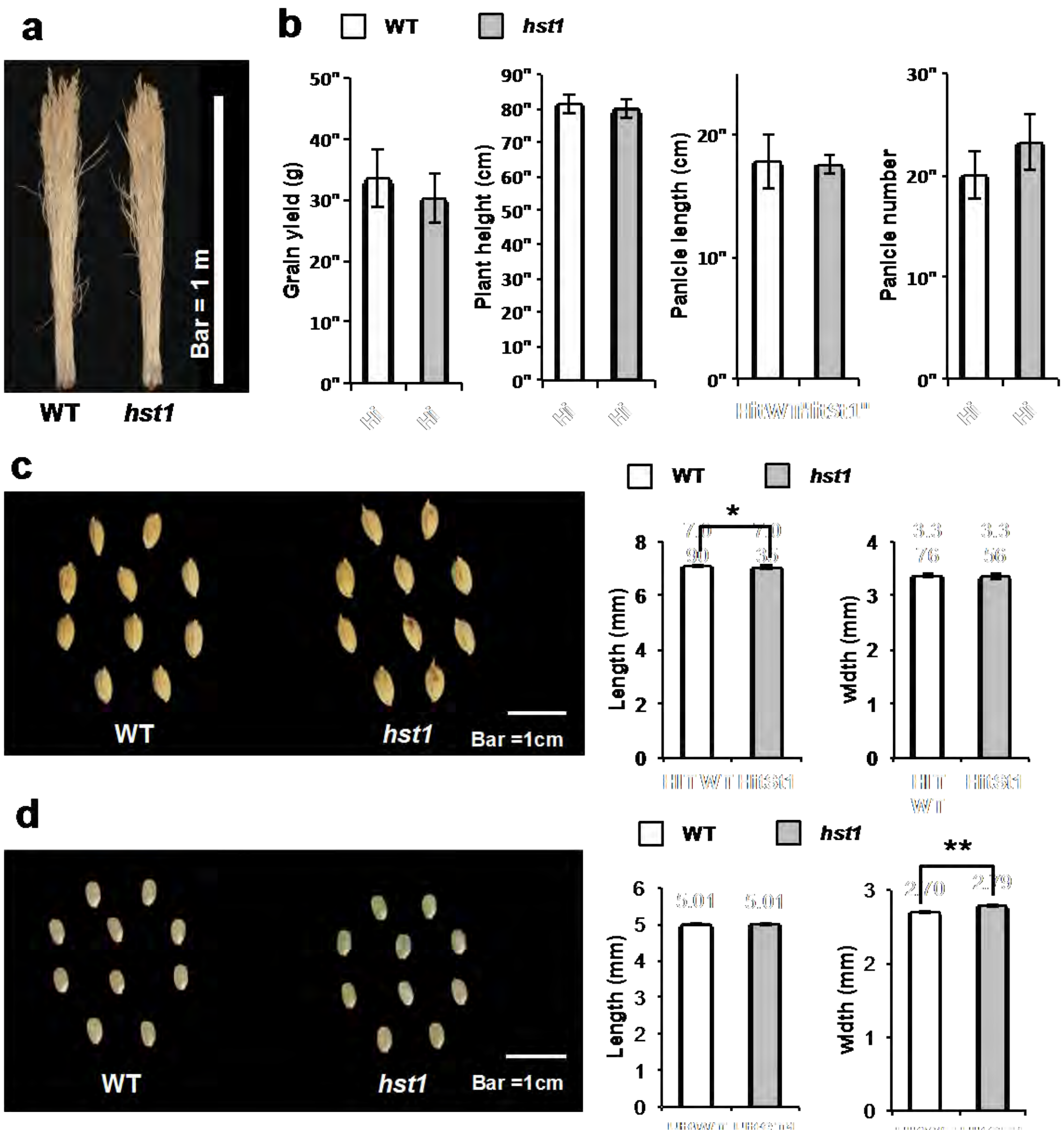
hst1 (1088)

Kaijin (201)

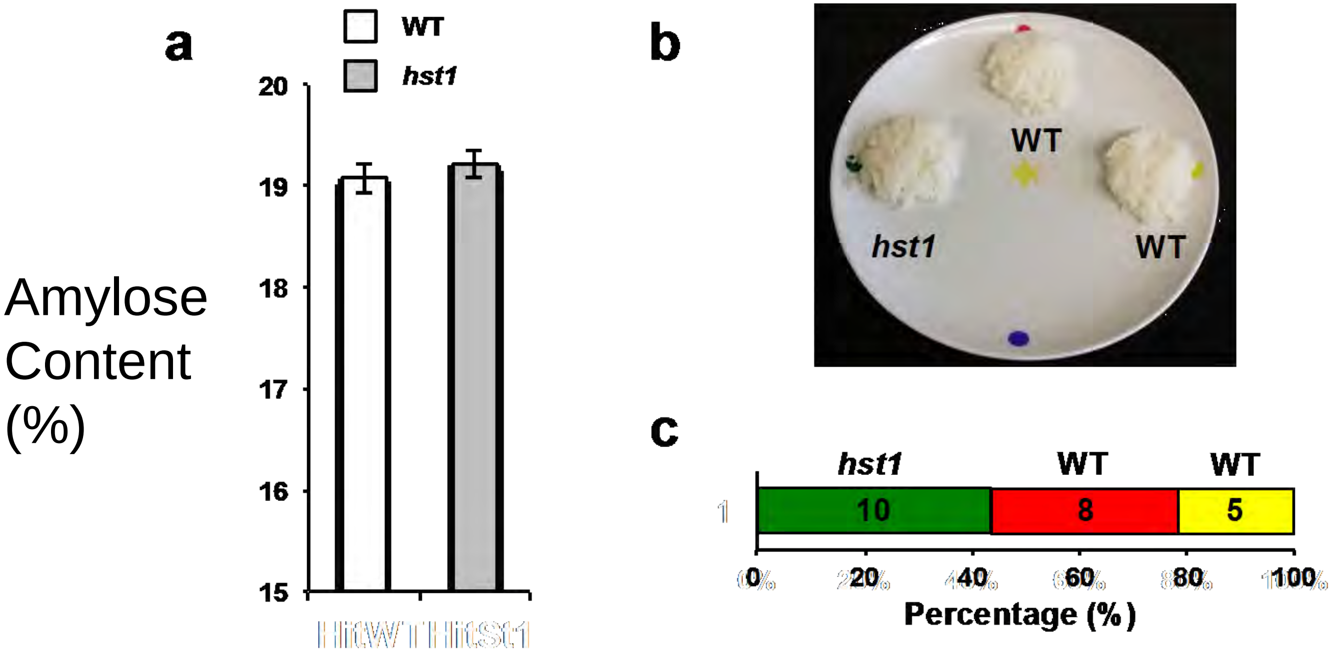


hst1

Kaijin is equivalent to Hitomebore in most traits



Kaijin has equal eating quality as Hitomebore



Eating quality blind test (23 panels)

A variety generated within 2 years
(cf. ~10 years conventional breeding)

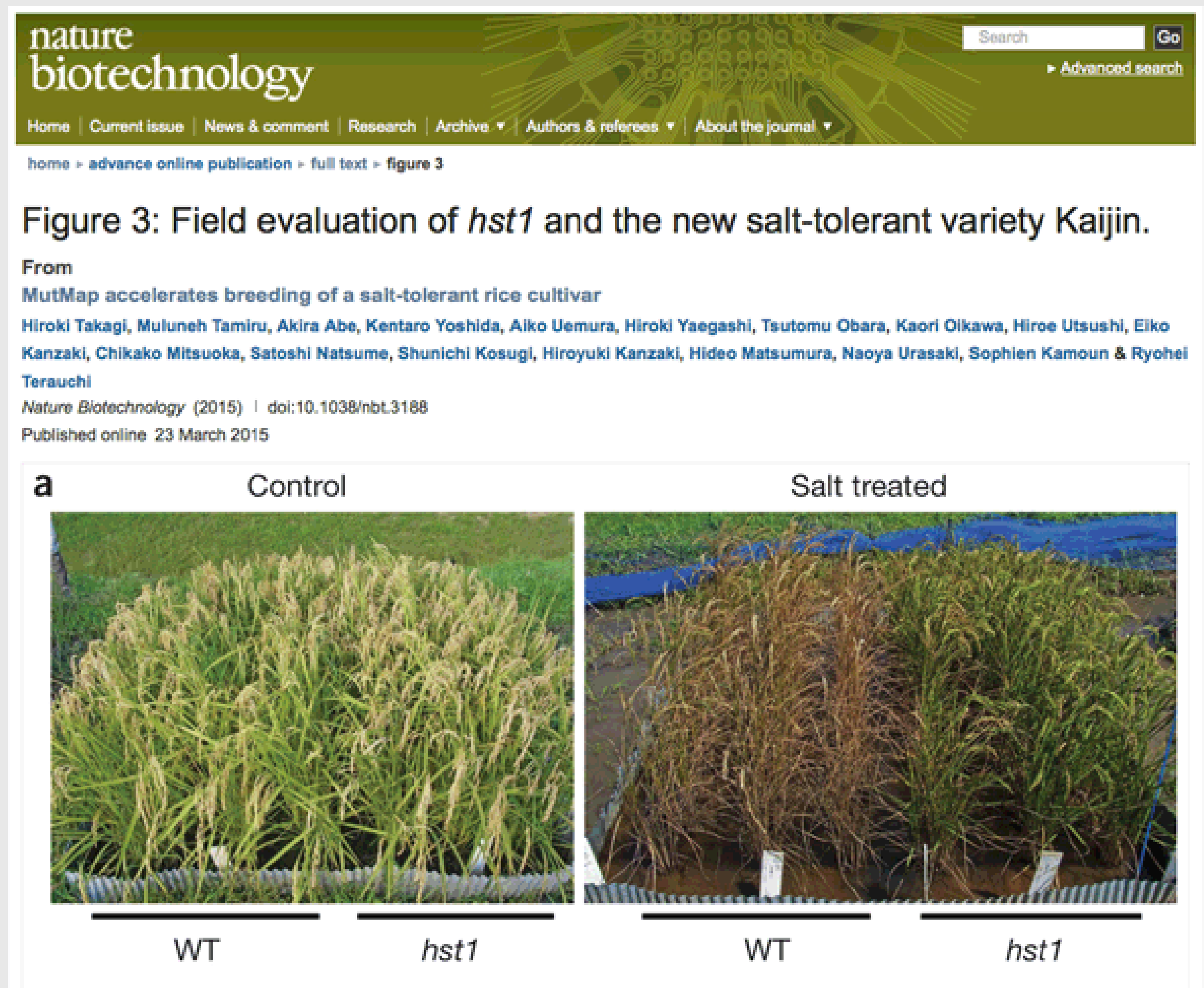


Figure 3: Field evaluation of *hst1* and the new salt-tolerant variety Kaijin.

From

MutMap accelerates breeding of a salt-tolerant rice cultivar

Hiroki Takagi, Muluneh Tamiru, Akira Abe, Kentaro Yoshida, Aiko Uemura, Hiroki Yaegashi, Tsutomu Obara, Kaori Oikawa, Hiroe Utsushi, Eiko Kanzaki, Chikako Mitsuoka, Satoshi Natsume, Shunichi Kosugi, Hiroyuki Kanzaki, Hideo Matsumura, Naoya Urasaki, Sophien Kamoun & Ryohei Terauchi

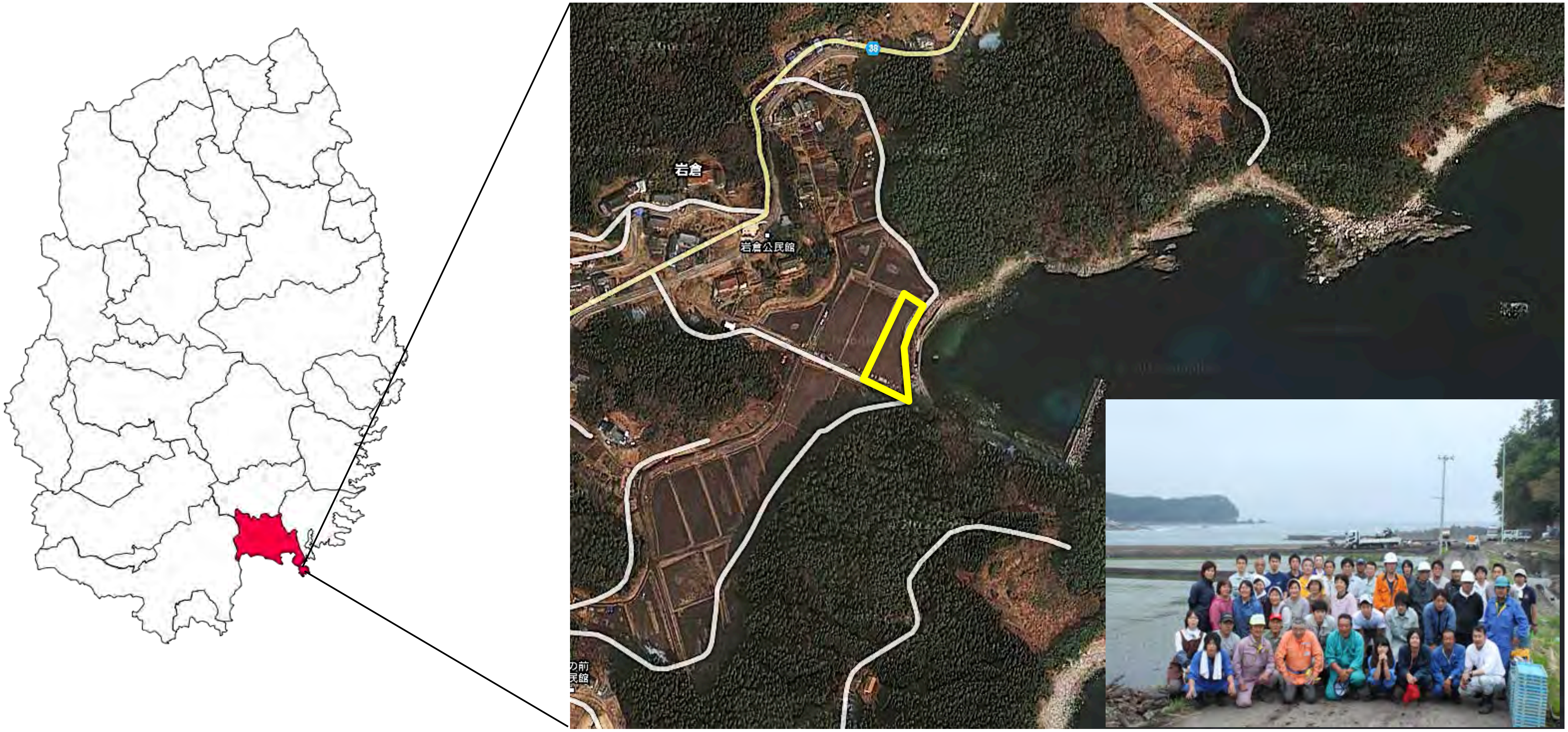
Nature Biotechnology (2015) | doi:10.1038/nbt.3188

Published online 23 March 2015

Takagi *et al.* 2015 Nature Biotechnol.

Trials in Tsunami-hit paddy at Rikuzen-Takata underway

Hirota, Rikuzentakata



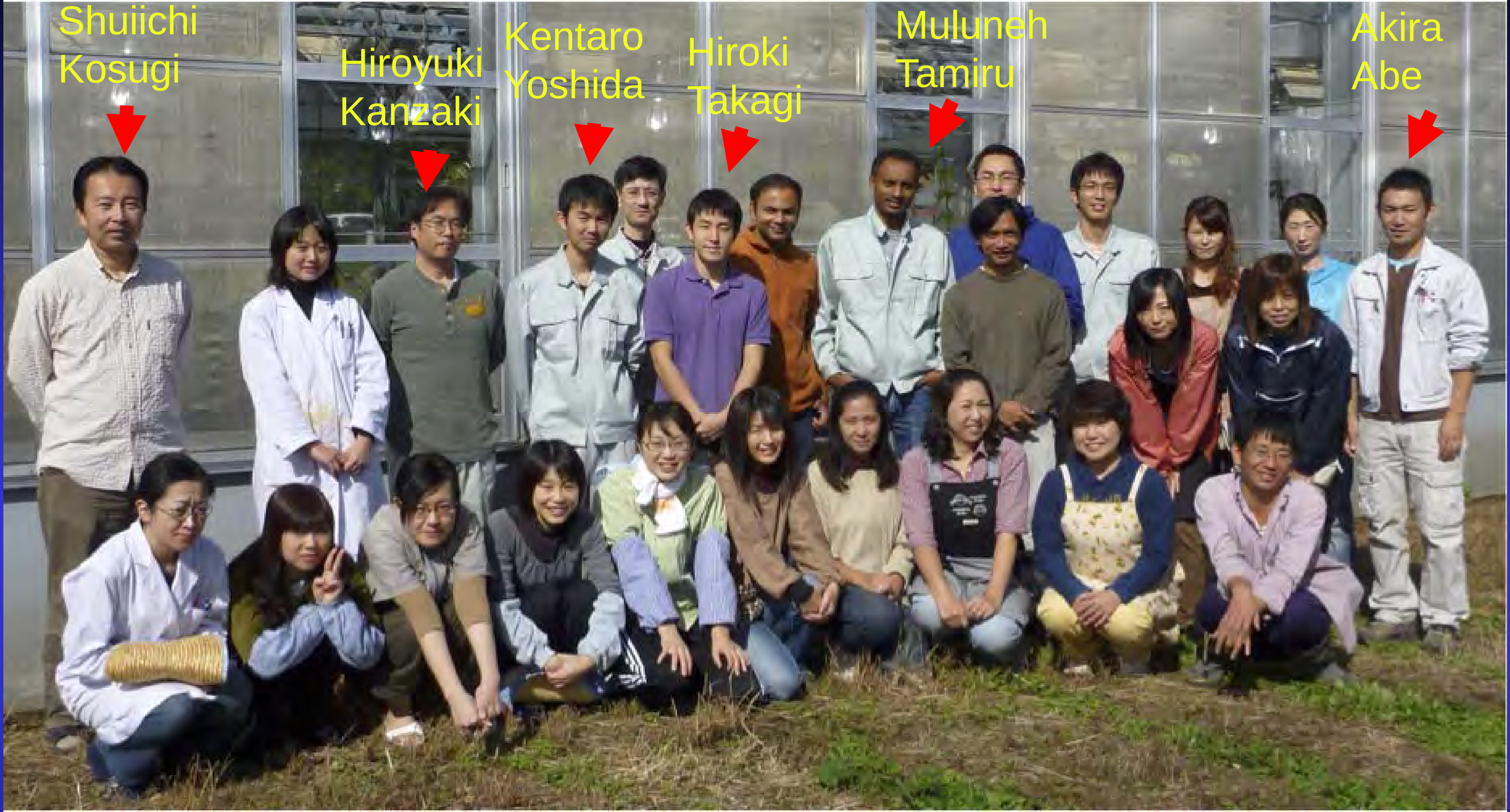
Genetic resources

x

Genome sequencing

Powerful tools for isolation of
important crop genes and rapid breeding

Bioinformatics pipelines
publicly available: http://www.ibrc.or



Coworkers:

Akira Abe, Hiroki Takagi, Kenrato Yoshida, Hiromas Saitoh, Hiroyuki Kanzaki, Hideo Matsumura, Muluneh Tamiru, Yudai Okuyama, Shunichi Kosugi, Satoshi Natsume

Acknowledgements

Collaborators:

Sophien Kamoun (Sainsbury Lab., UK)

Naoya Urasaki (Okinawa Agricultural Res. Center, Japan)

Hideo Matsumura (Shinshu University, Japan)

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JSPS

PROBRAIN, Japan; MAFF, Japan

MEXT, Japan; IITA, Nigeria