

**Regional Training Course on Plant Mutation Breeding and Molecular Techniques for Crop Improvement**

**1 May, 2024**

**Topic: MutMap + QTL-seq**



## Rice cultivar “Hitomebore”

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Major elite cultivar of Tohoku region

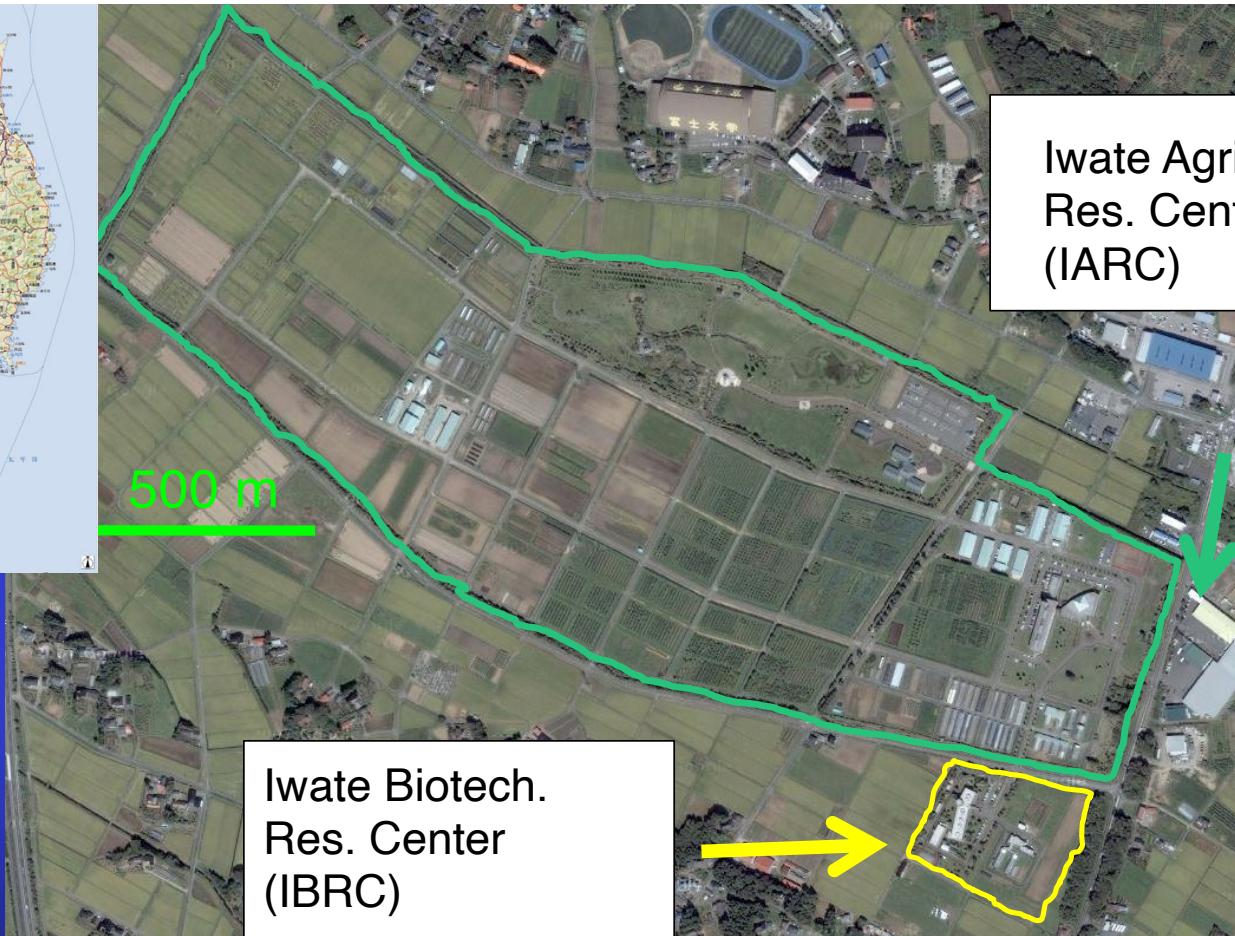
Improvements needed:

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

→ Deliver **safer** and  
more **competitive** cultivars to farmers



Kitakami  
Iwate



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

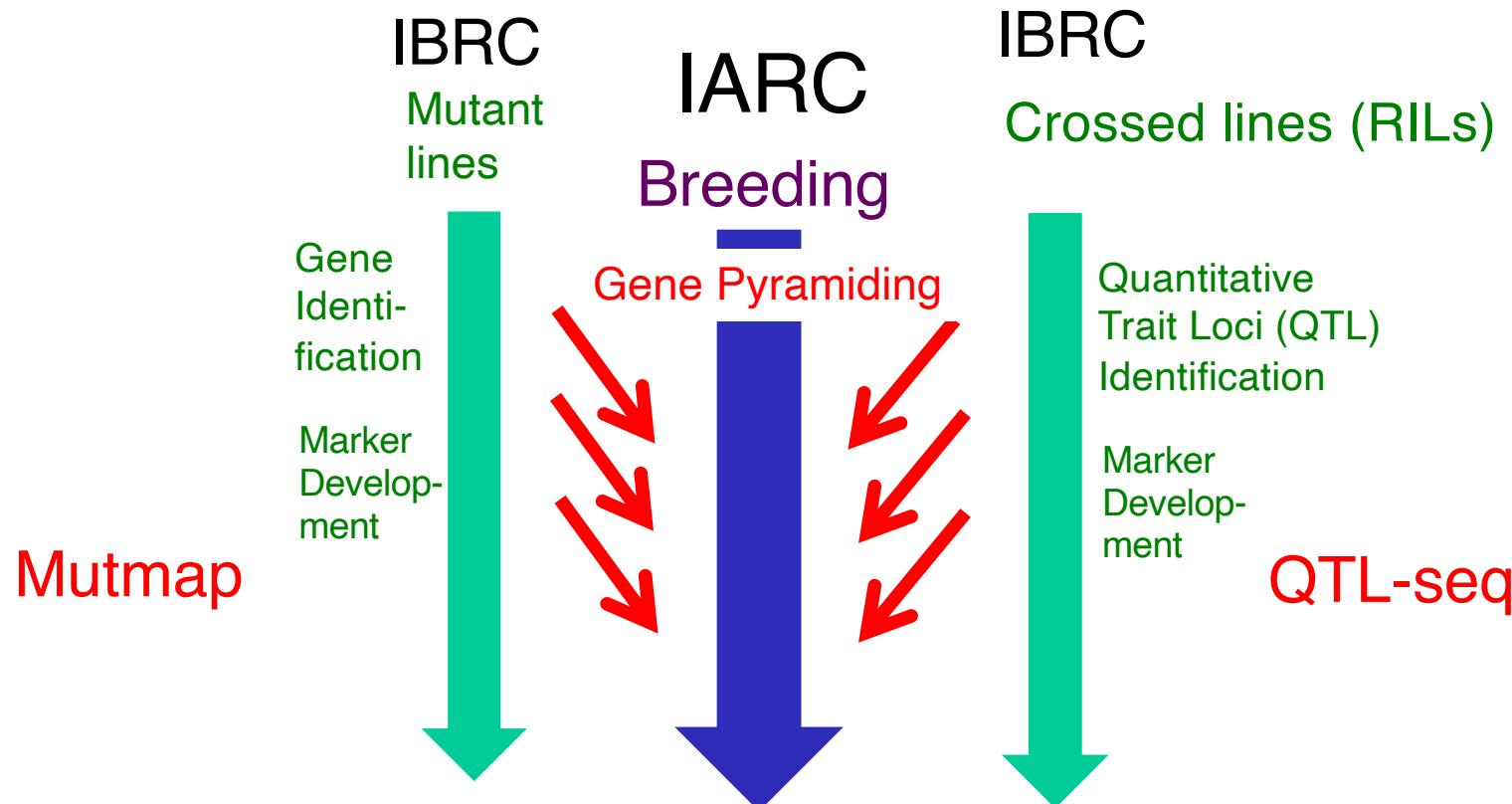


# Rice Breeding in Iwate

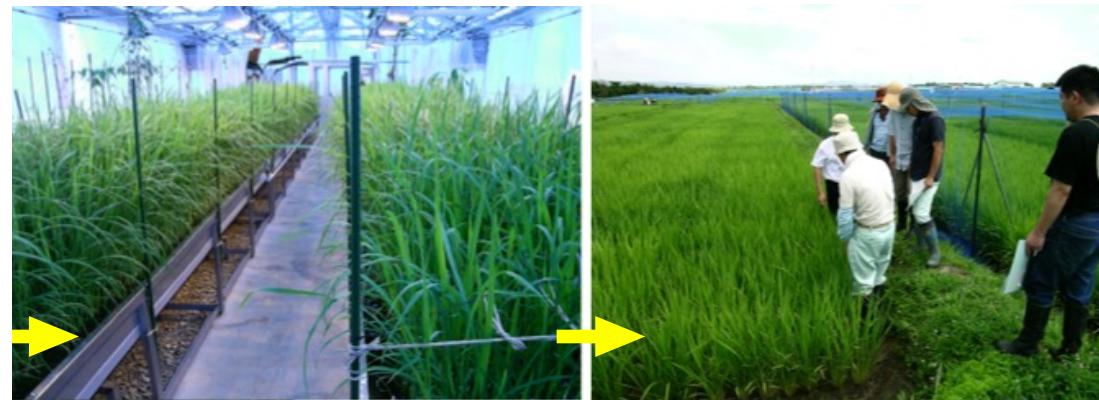
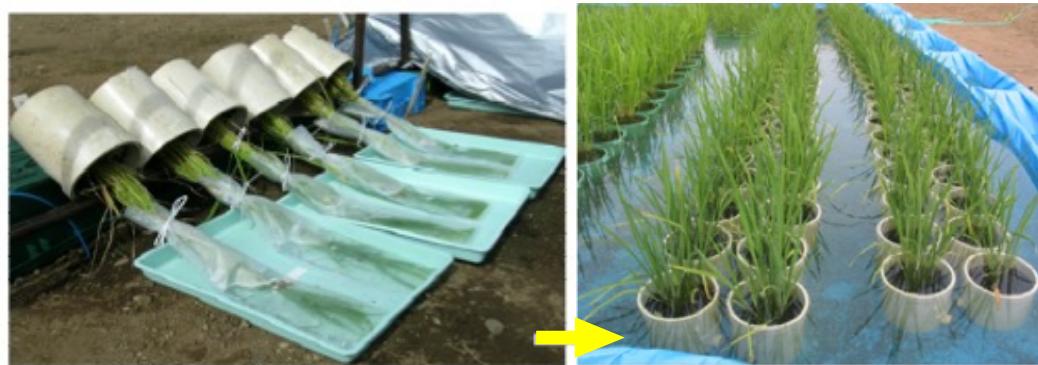
Elite cultivar  
“Hitomebore”

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

## Non transgenic



# EMS mutagenesis of Hitomebore



12,000 M3-M5 lines

~1,500 Single Nucleotide Polymorphisms (SNPs / line)

## EMS (ethanemethylsulfonate) mutagenesis

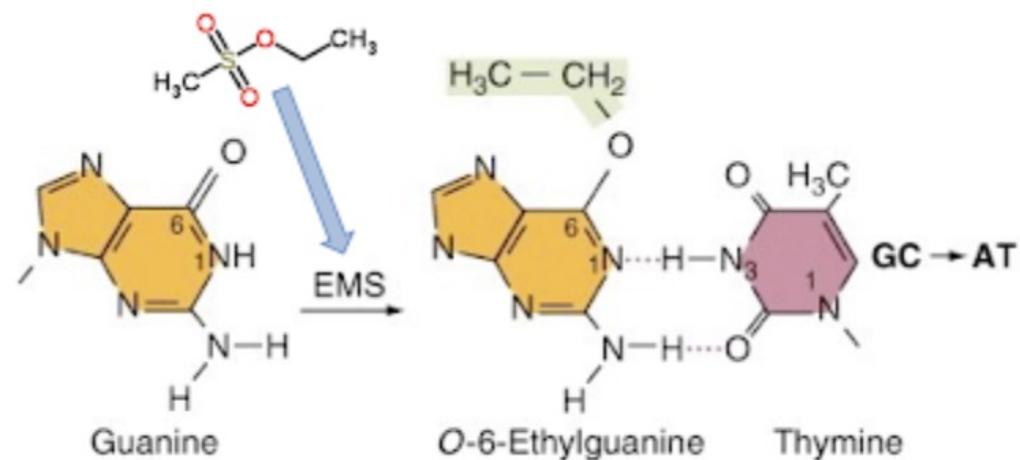


Figure adapted from Griffiths – An Introduction to Genetic Analysis

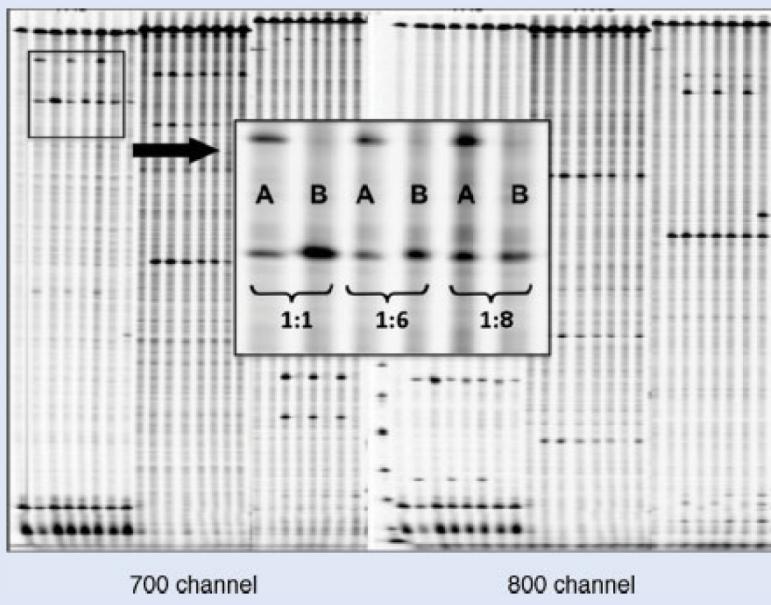
Source:

<http://rrresearch.fieldofscience.com/2013/09/a-better-strategy-for-finding-new.html>

# Use of rice mutant lines for TILLING

## Bulking of DNA in Rice TILLING

The standard TILLING protocol mixes reference and subject DNA in a 1 : 1 ratio. Screening large numbers of DNAs for the purpose of mutation detection in this



*The Handbook of Plant Mutation Screening*. Edited by Günter Kahl and Khalid Meksem  
Copyright © 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim  
ISBN: 978-3-527-32604-4

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)

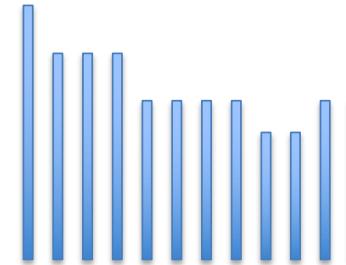


Data management by bar-codes

# Rice genome

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12 chromosomes

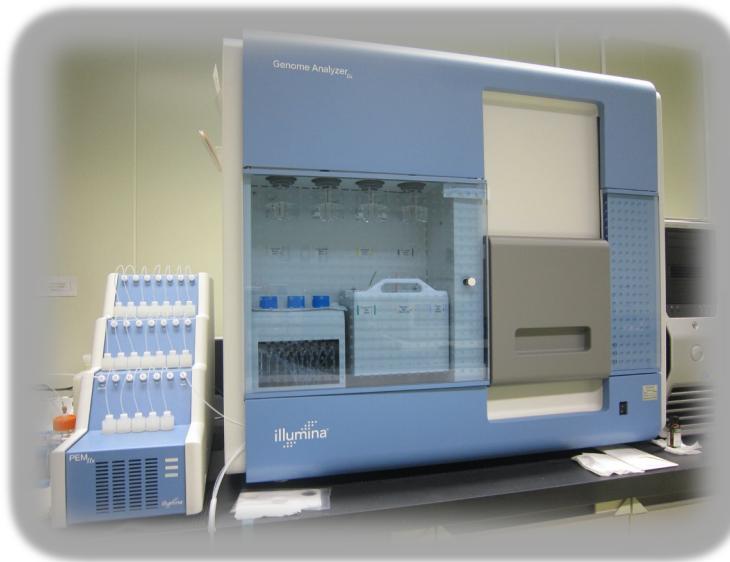


- Accurate reference genome sequence (cv. Nipponbare)  
published in 2005
- 389 Mb = 389 million bases (nucleotides)
- 38,000 protein coding genes



Gene function largely  
unknown

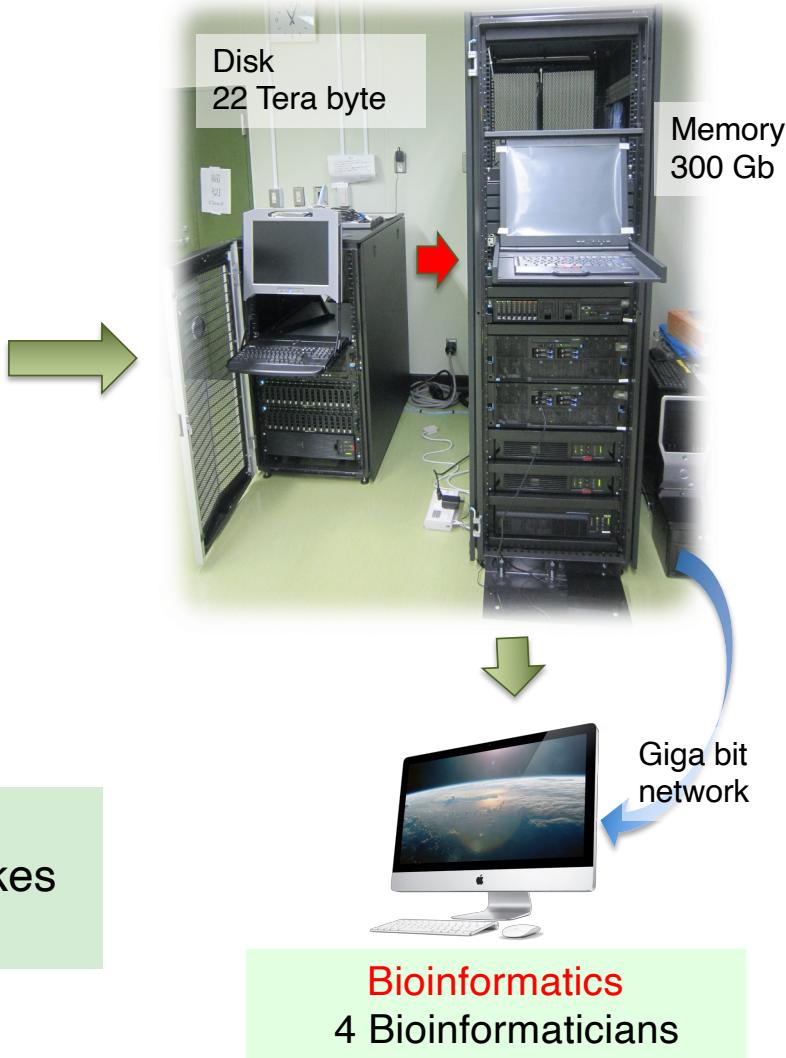
## IBRC next generation sequencing (NGS) platform (2010-)



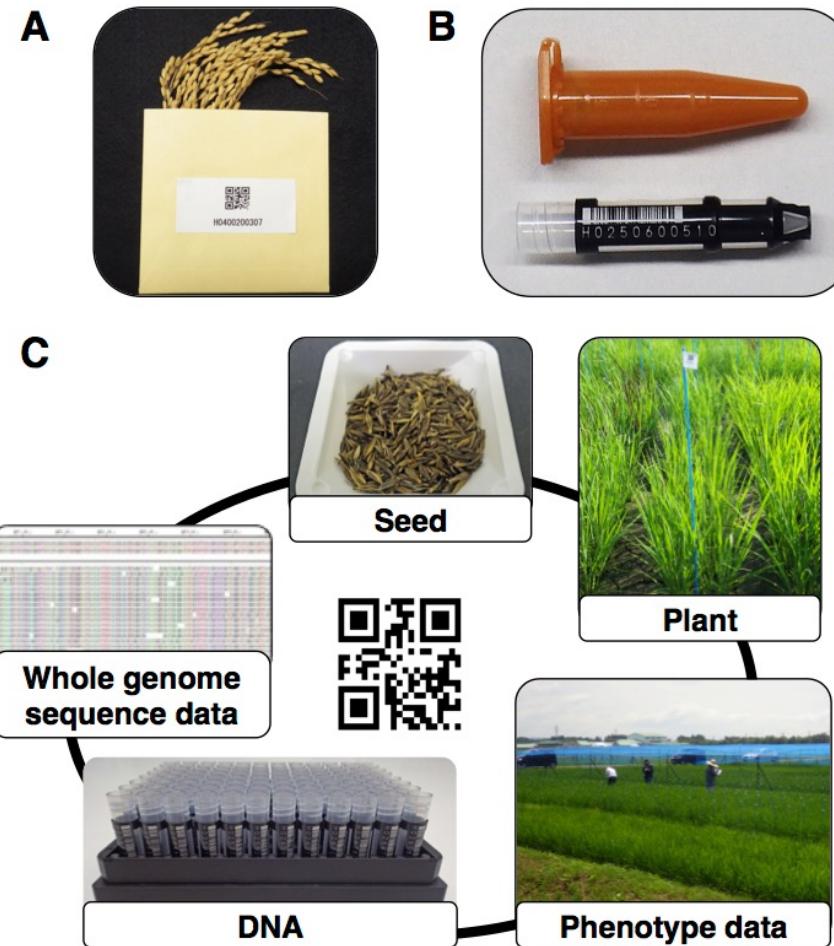
Illumina Genome Analyzer IIx  
(GAIIX)

"Most widely  
adopted NGS system"

50 Gb  
A single run takes  
~ 7 days



# Genetic resource is the key



# 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<sup>1,2,7</sup>, Shunichi Kosugi<sup>3,7</sup>, Kentaro Yoshida<sup>3</sup>, Satoshi Natsume<sup>3</sup>, Hiroki Takagi<sup>2,3</sup>, Hiroyuki Kanzaki<sup>3</sup>, Hideo Matsumura<sup>3,4</sup>, Kakoto Yoshida<sup>3</sup>, Chikako Mitsuoka<sup>3</sup>, Muluneh Tamiru<sup>3</sup>, Hideki Innan<sup>5</sup>, Liliana Cano<sup>6</sup>, Sophien Kamoun<sup>6</sup> & Ryohei Terauchi<sup>3</sup>

The majority of agronomic traits are controlled by multiple genes of these genes difficult. Here we introduce MutMap, a method by 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 it mutant F<sub>2</sub> progeny that are required. We applied MutMap to several unique genomic positions most probable to harbor mutations causing relevant trait. These results show that MutMap can accelerate th

ture America, Inc. All rights reserved.

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<sup>1</sup>. 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 changes. Crop breeding is important for improving yield and toler



Akira Abe



Shunichi  
Kosugi

## MutMap: Background

### Conventional map-based cloning of crop genes

**Hitomebore**

**mutant            ×        *indica* rice**

**(*japonica*)**

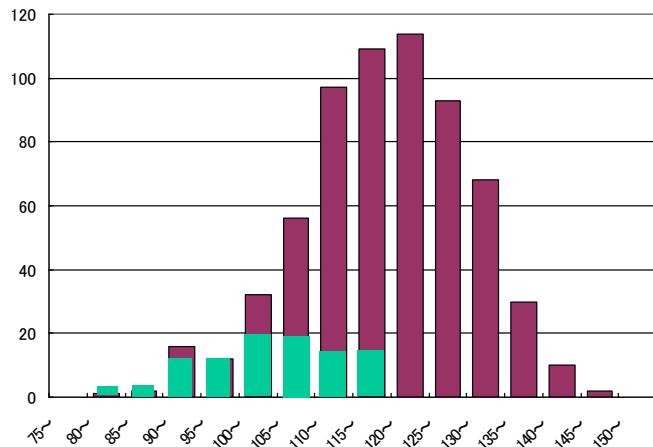
↓

**F<sub>2</sub> segregants**

- ① **Needs a large number of DNA markers**
  - **Use a distantly related line for crossing**  
**(*indica*)**
- ② **Needs a large number of recessive homozygotes**
  - **Easy-to-score characters**
- ③ **Needs to develop DNA markers**
  - **Cost and Time**



## MutMap: Background

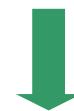


**Hitomebore (*japonica*) x Kasalath (*indica*)**  
**642 F2 individuals**  
**Distribution of Plant Height**

**Can you select recessive homozygote individuals from F2 ?**

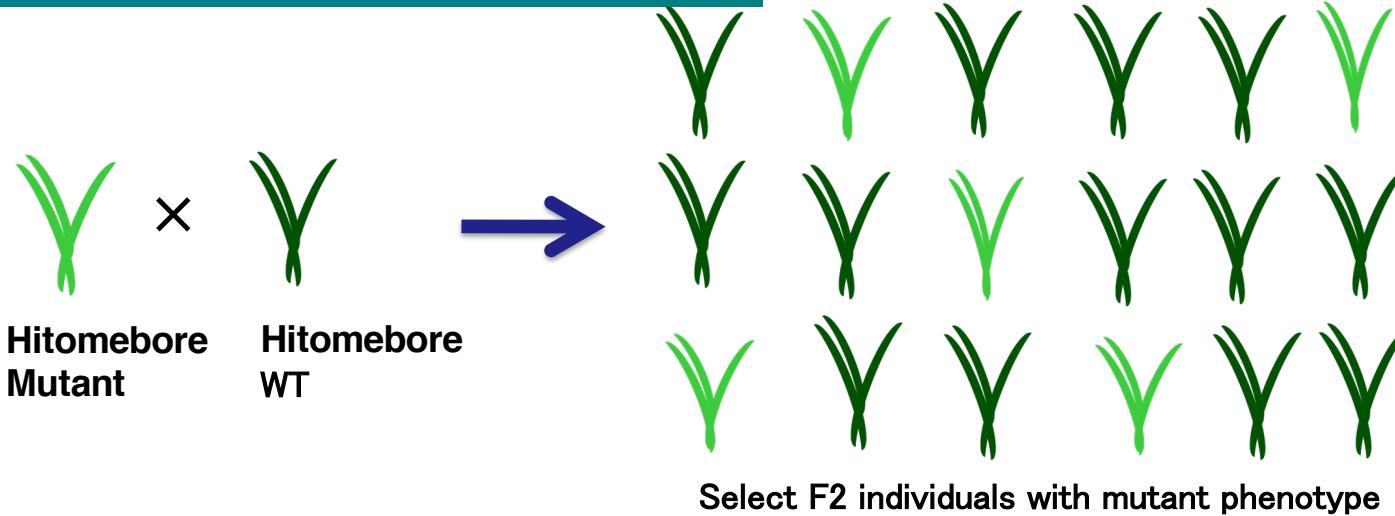


**Semi-dwarf, Starch Quality, Field Resistance etc.**



**Not easy!**

## MutMap: crossing mutant to WT parental line

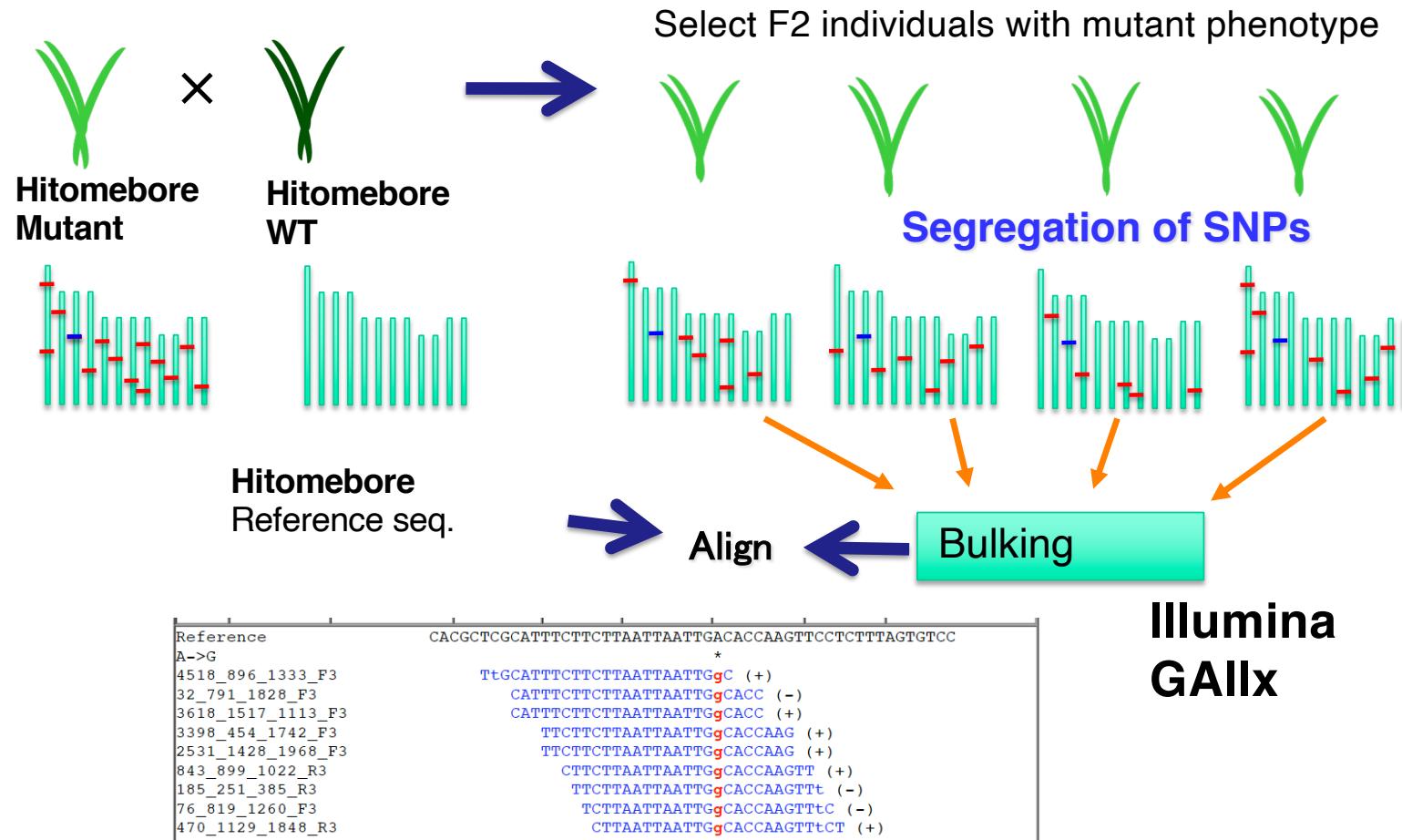


**Point: Cross to the parental WT line**

**Clear segregation of phenotype in F2**



## MutMap: Bulk sequencing of F2



## Principle

### ① SNP calling of bulked DNA : SNP-index

Hitomebore WT = Reference

ACGCTCGCATTCTTCTTAATTAATTGACACCAAGTTCCCTTTAGTGT  
\*  
TtGCATTTCTTCTTAATTAATTGgC (+)  
CATTTCTTCTTAATTAATTGgCACC (-)  
CATTTCTTCTTAATTAATTGgCACC (+)  
TTCTTCTTAATTAATTGgCACCAAG (+)  
TTCTTCTTAATTAATTGACACCAAG (+)  
CTTCTTAATTAATTGACACCAAGTT (+)  
TTCTTAATTAATTGACACCAAGTT (-)  
TCTTAATTAATTGACACCAAGTTc (-)  
CTTAATTAATTGACACCAAGTTtCT (+)  
CTTAATTAATTGACACCAAGTTtCT (+)

4 / 10 reads show “g”

SNP-index = 0.4

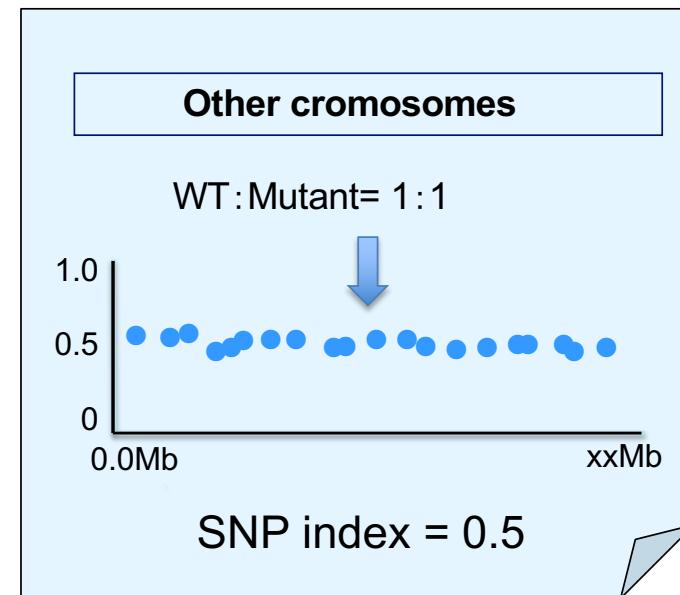
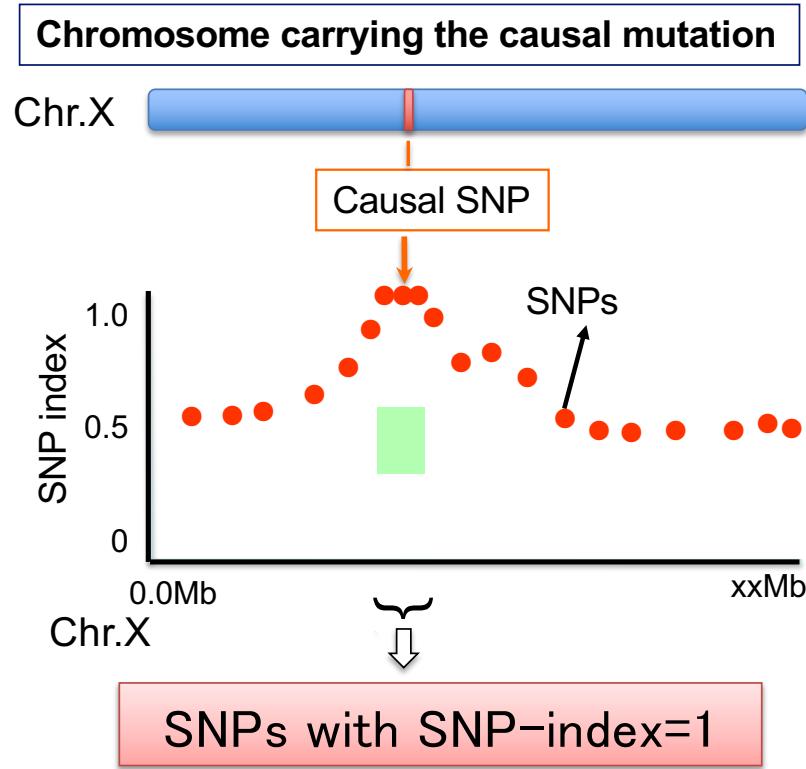
ACGCTCGCATTCTTCTTAATTAATTGACACCAAGTTCCCTTTAGTGT  
\*  
TtGCATTTCTTCTTAATTAATTGgC (+)  
CATTTCTTCTTAATTAATTGgCACC (-)  
CATTTCTTCTTAATTAATTGgCACC (+)  
TTCTTCTTAATTAATTGgCACCAAG (+)  
TTCTTCTTAATTAATTGgCACCAAG (+)  
CTTCTTAATTAATTGgCACCAAGTT (+)  
TTCTTAATTAATTGgCACCAAGTT (-)  
TCTTAATTAATTGgCACCAAGTTc (-)  
CTTAATTAATTGgCACCAAGTTtCT (+)  
CTTAATTAATTGgCACCAAGTTtCT (+)

10 / 10 reads show “g”

SNP-index = 1.0

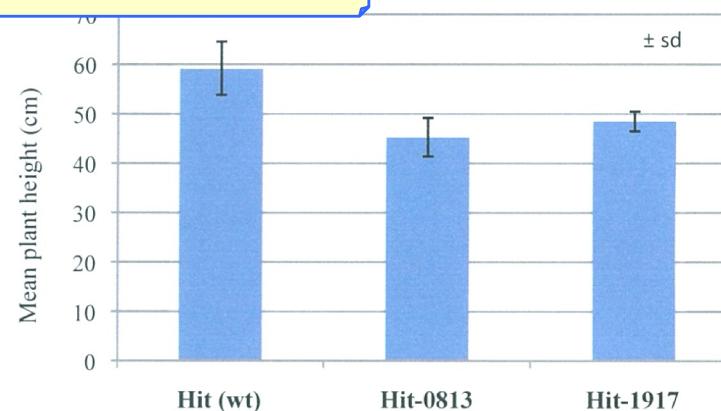
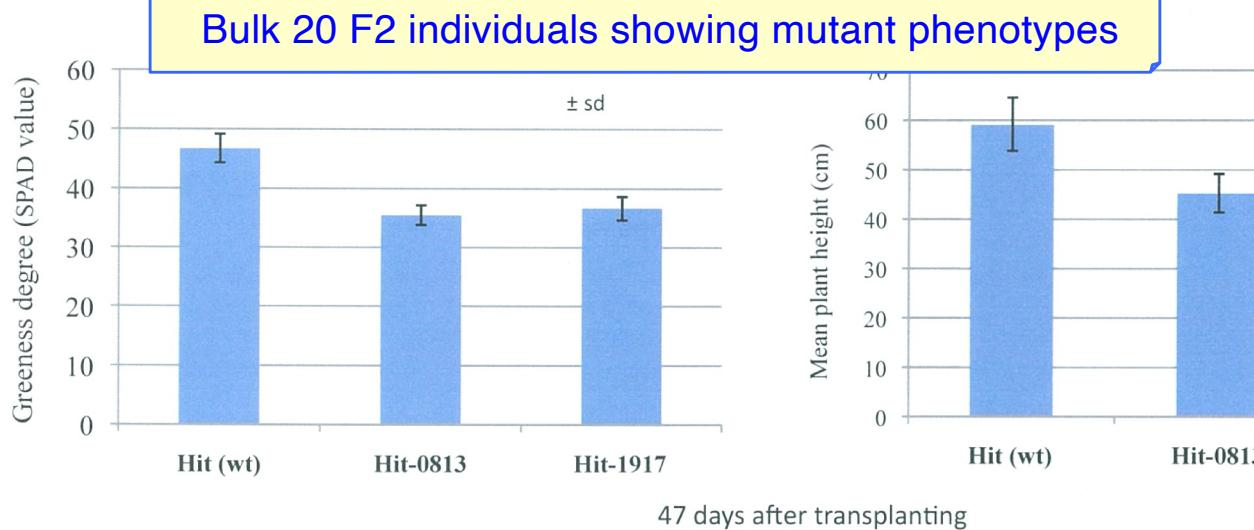
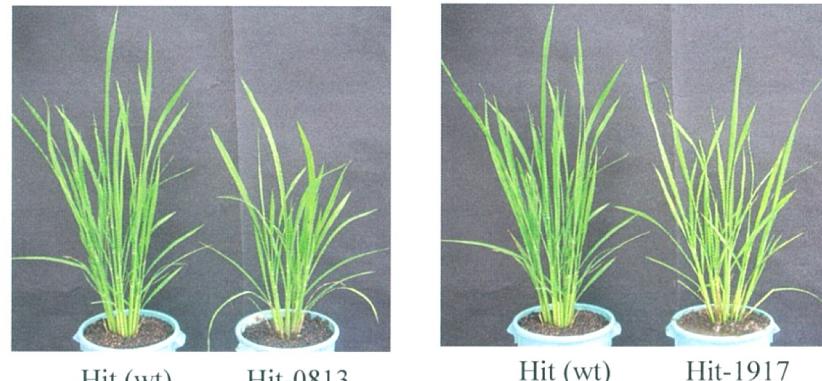
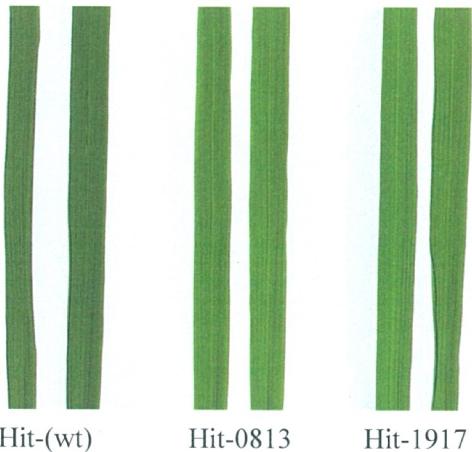
# Principle

Plot SNP-index across the genome

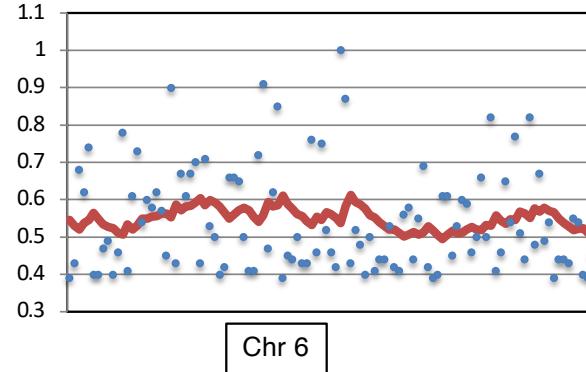
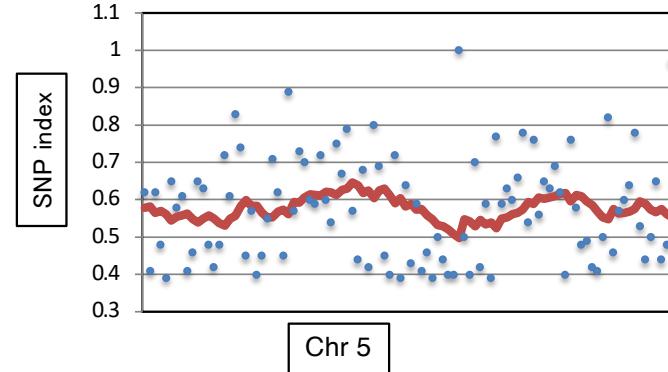
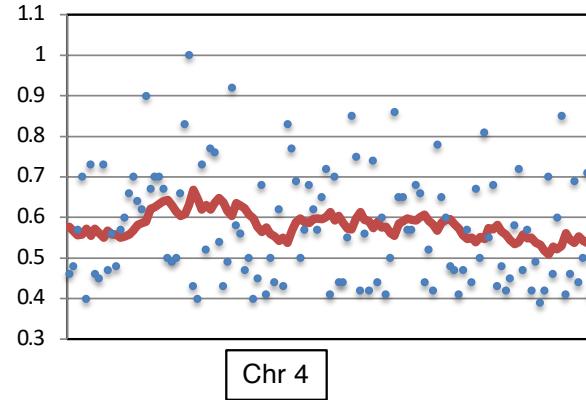
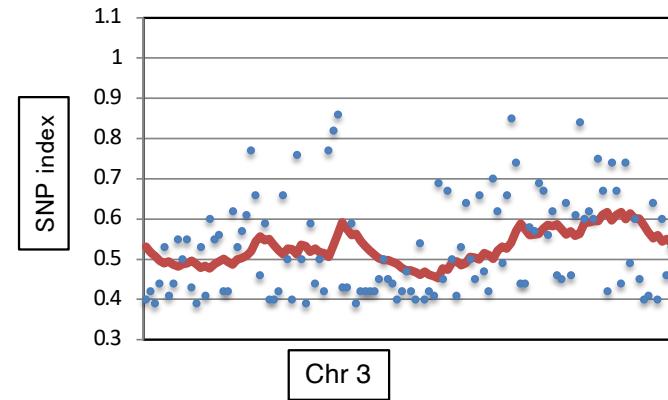
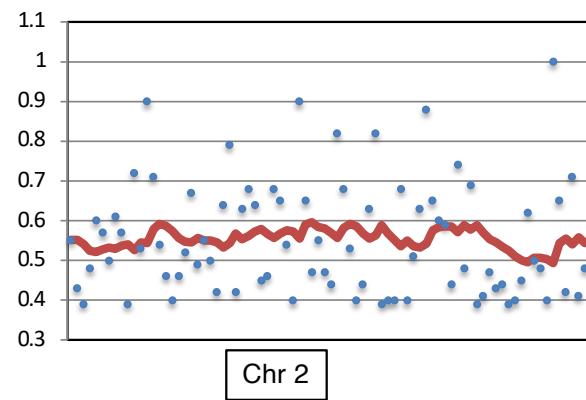
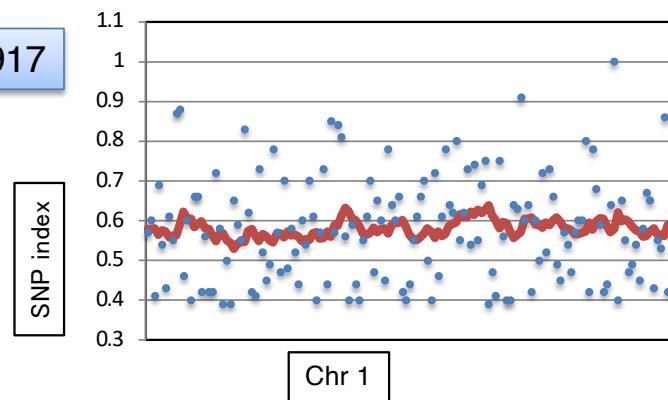


## Proof of Principle

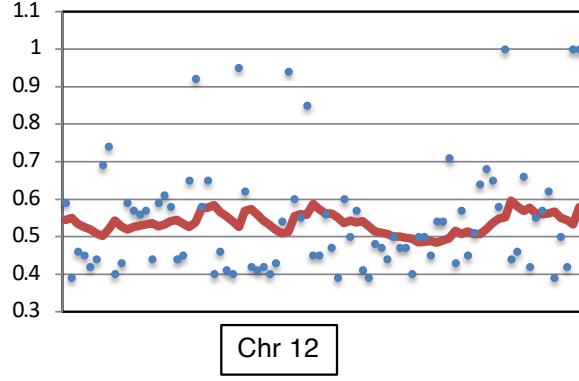
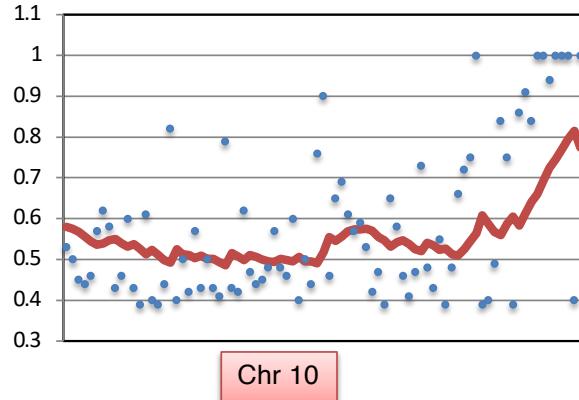
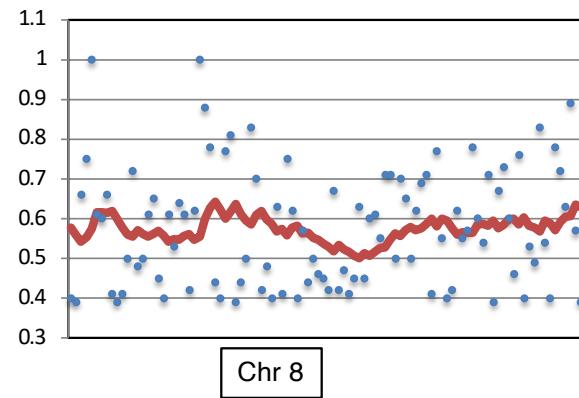
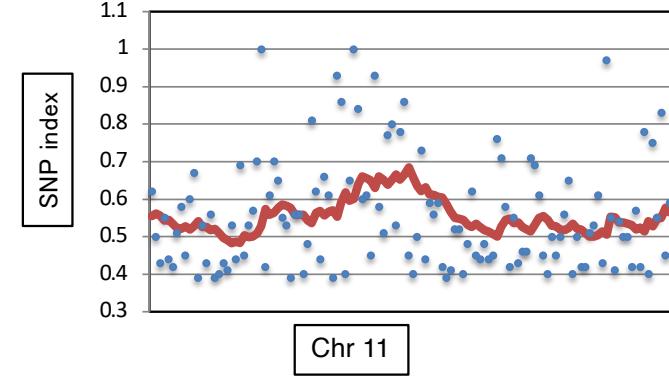
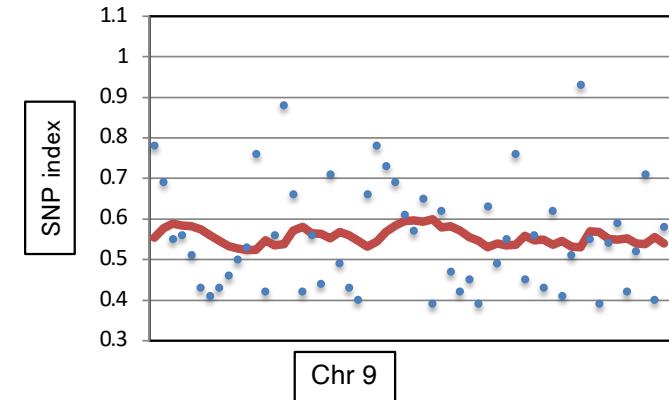
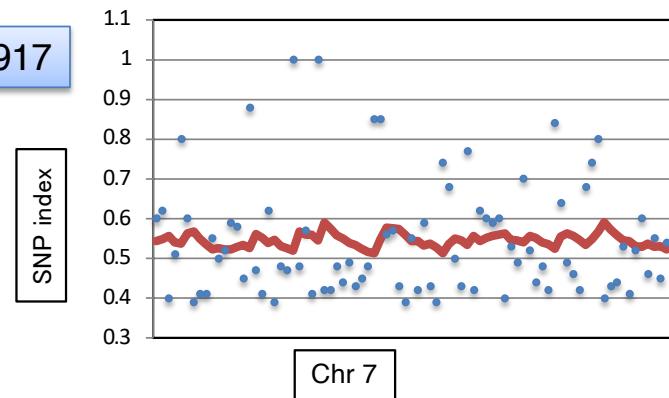
### Hit-0813 and Hit-1917 mutants



Hit1917

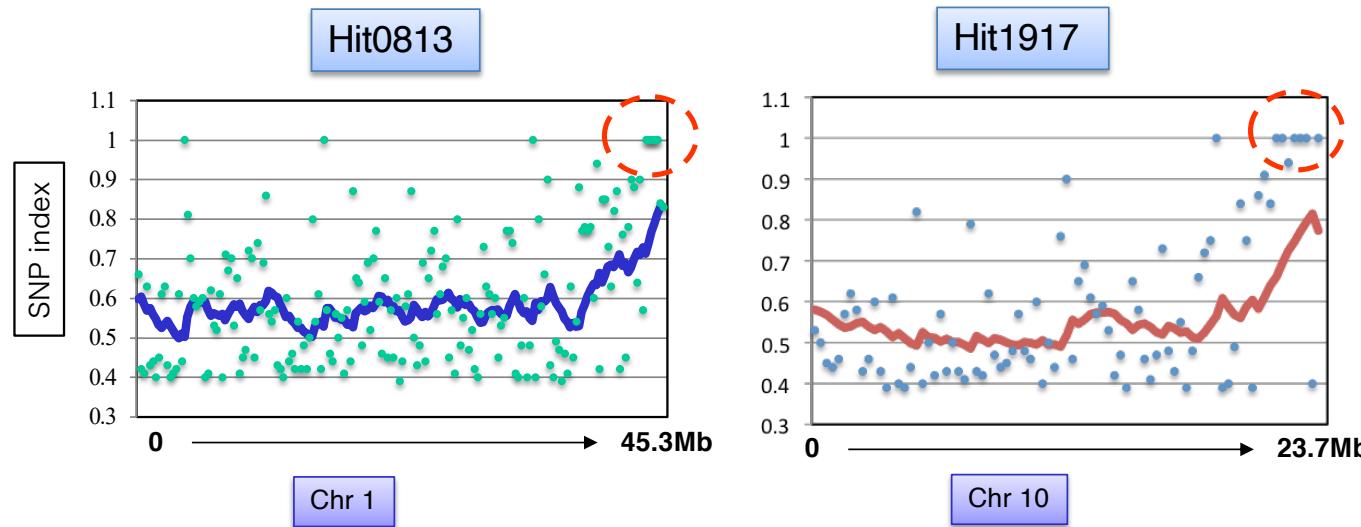


**Hit1917**



## Proof of principle

Genomic regions showing  
SNP-index  $\approx 1$



## Proof of principle

### Genomics regions with SNP-index $\approx 1$

Hit0813: Chr. 1

Position	Cover.	SNP index
42979297	10	0.78
43103113	7	0.55
43273384	10	0.9
43273451	9	0.88
43390581	17	0.64
43488206	12	0.9
43814878	11	1.0
44003965	10	1.0
44051208	11	1.0
44447768	8	1.0
44611967	11	1.0
44711612	13	0.84
44890284	7	0.83

Hit1917: Chr. 10

Position	Cover.	SNP index
20083465	12	0.5
20568498	99	0.84
20996171	8	0.75
21526433	17	0.86
22179795	15	0.91
22192527	13	0.84
22229793	11	1.0
22455081	11	1.0
22572645	19	0.94
22573263	11	1.0
22825487	12	1.0
22981826	15	1.0
23648959	16	1.0

## Proof of principle

### Genes in the identified regions

Hit0813: Chr 1 : 43488206~44611967

SNP (43488206)  
pyrroline-5-carboxylate reductase-like gene : intron

SNP (44003965)  
Signal recognition particle 2C (SRP54 subunit 2C) GTPase gene: intron

Hit1917: Chr 10 : 22229793~23648959

SNP (22981826)  
Chlorophyllide a oxygenase 2C isoform2-like (526 aa)  
Gene: exon

*OsCAO1*

253<sup>th</sup> Leu → Phe

SNP (23202531)  
Zinc finger, RING/FYVE/PHD-type domain protein  
Gene: exon

106<sup>th</sup> Ala → Val

SNP (23648959)  
S/T-kinase domain protein gene: intron

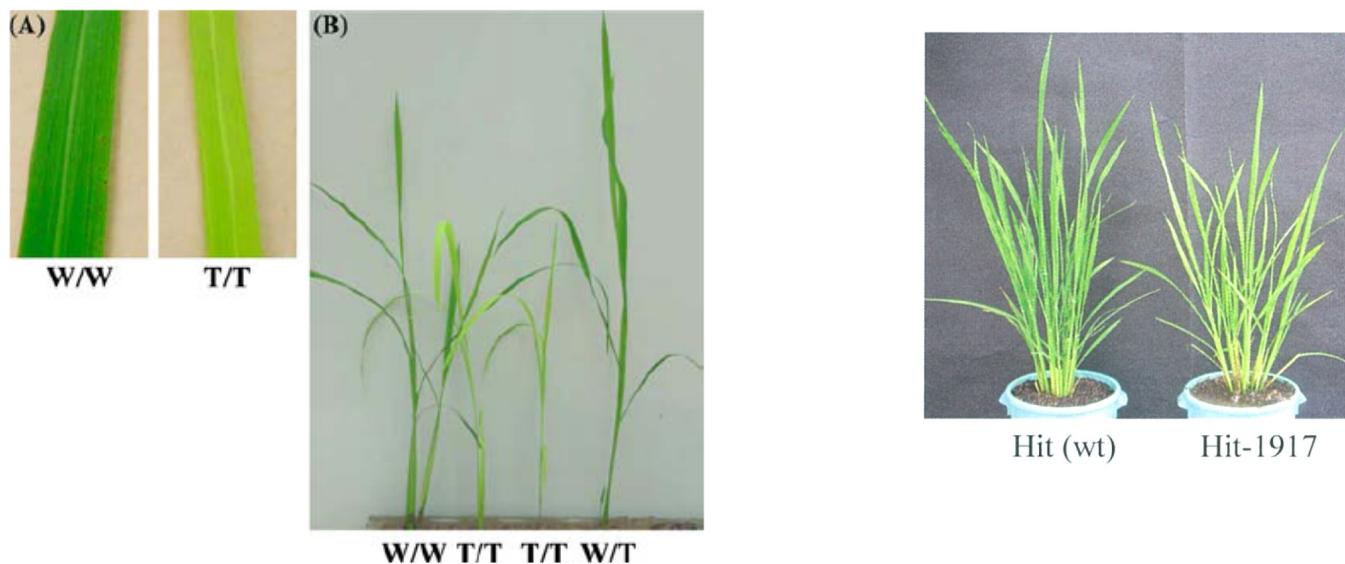
# Proof of principle

Plant Molecular Biology (2005) 57:805–818  
DOI 10.1007/s11103-005-2066-9

## Differential regulation of *chlorophyll a oxygenase* genes in rice

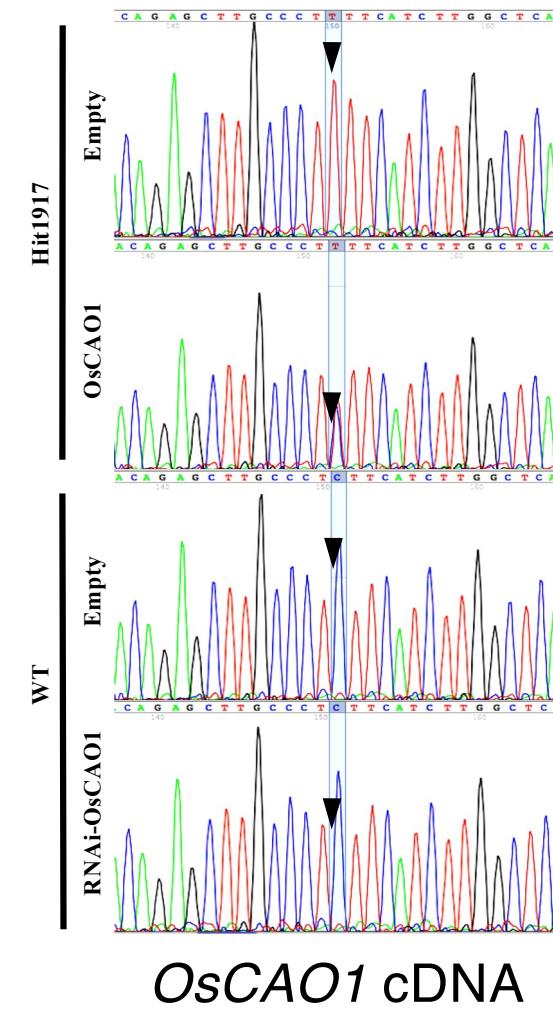
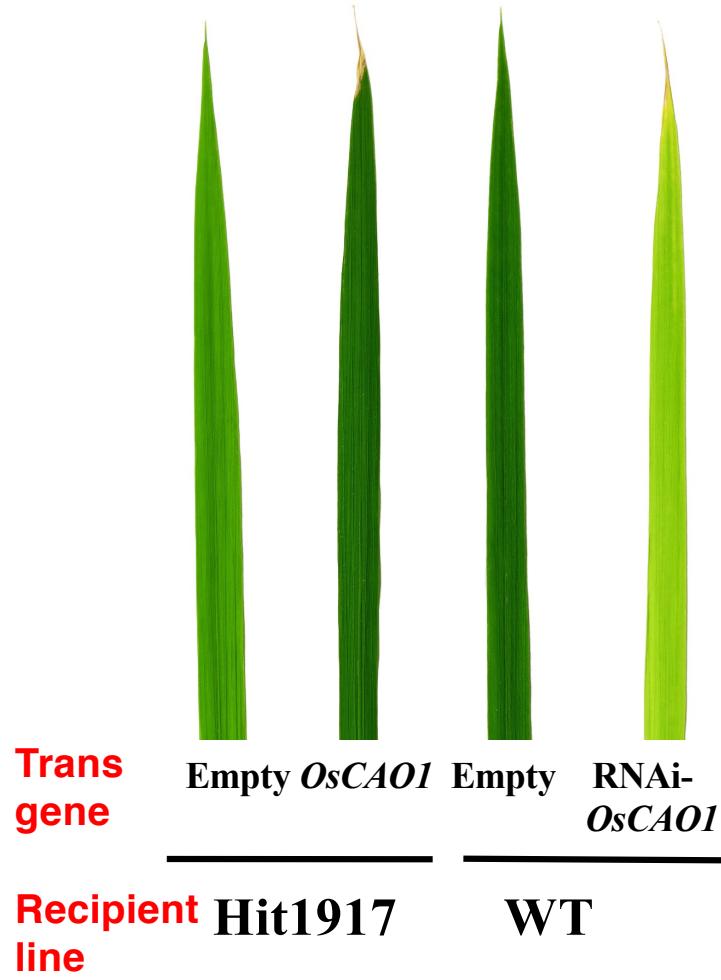
*OsCAO1*

Sichul Lee<sup>1</sup>, Jin-Hong Kim<sup>2</sup>, Eun Sang Yoo<sup>2</sup>, Choon-Hwan Lee<sup>2</sup>, Hirohiko Hirochika<sup>3</sup>  
and Gynheung An<sup>1,\*</sup>

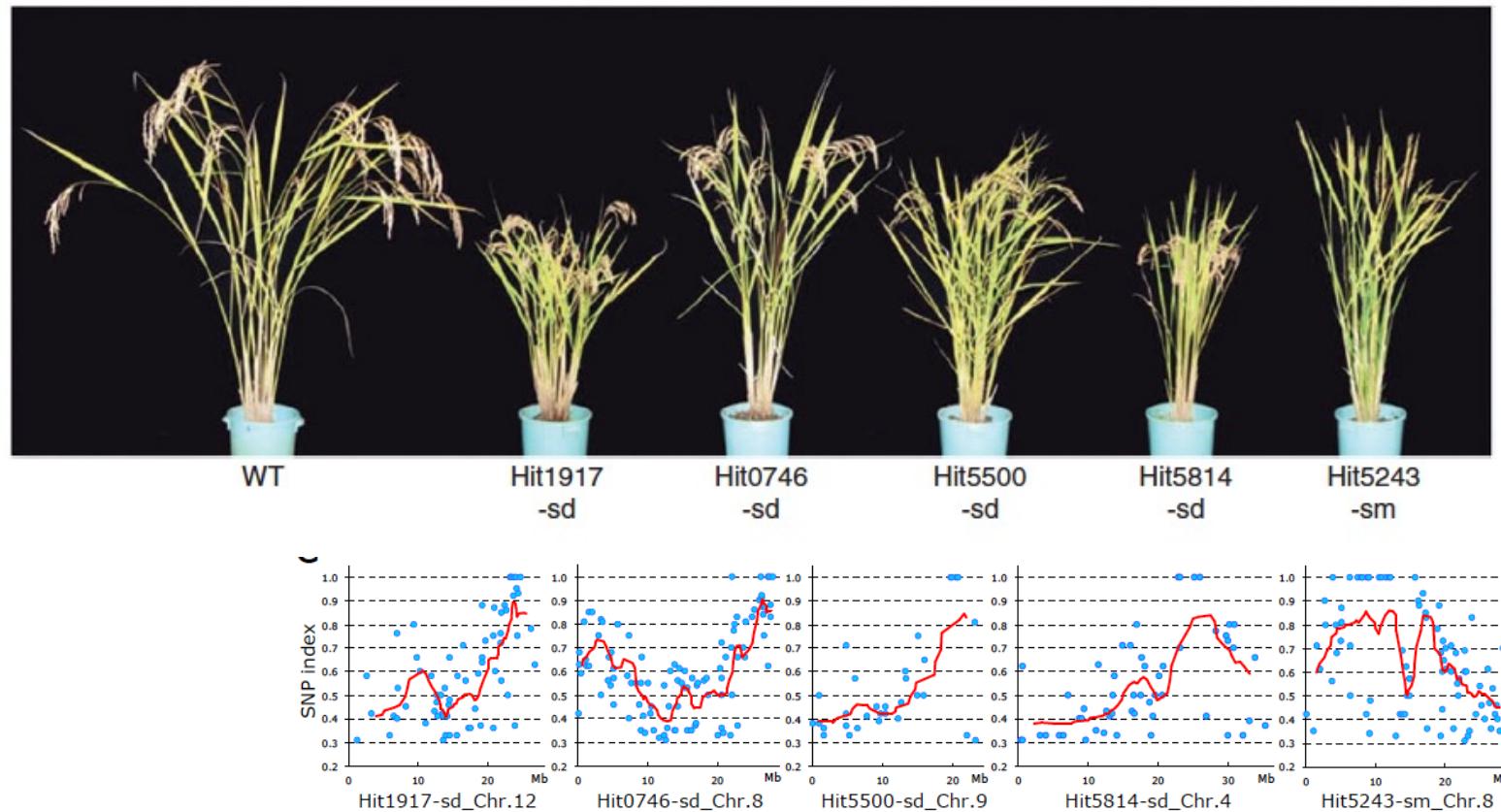


*Figure 6.* Phenotype comparisons between *OsCAO1* KO and WT plants at 30 DAG. T/T, homozygous mutant plant; W/W, wild-type segregant from tagged line; W/T, heterozygous plant.

# Complementation of Hit1917 phenotype with *OsCAO1*

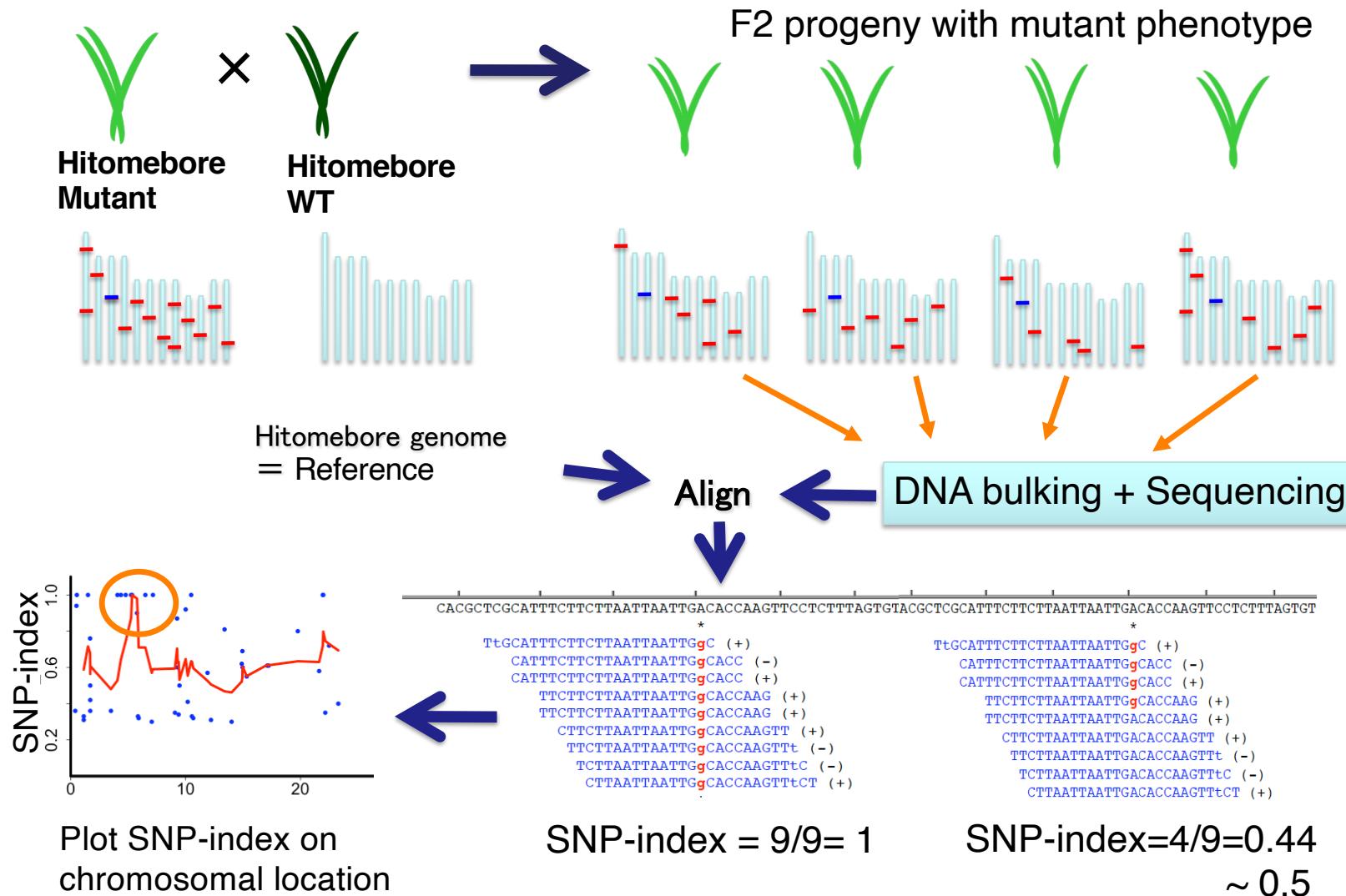


## MutMap applied to 4 semi-dwarf + 1 male sterile mutants



# MutMap method

Abe et al. Nat. Biotech. 2012



## MutMap

### Map-based cloning

Hitomebore Mutant × Indica rice



F<sub>2</sub> segregants

- ① Needs a large number of DNA markers
  - Cross to a distantly related rice lines
- ② Needs many F<sub>2</sub> individuals with mutant phenotype
  - Easy to score phenotypes
- ③ Needs fine mapping
  - Cost and Time

### MutMap

Hitomebore Mutant × Hitomebore WT

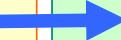


F<sub>2</sub> segregants

Gene can be isolated  
20 - 50 F<sub>2</sub> individuals

Whole genome sequencing

Allows quick breeding

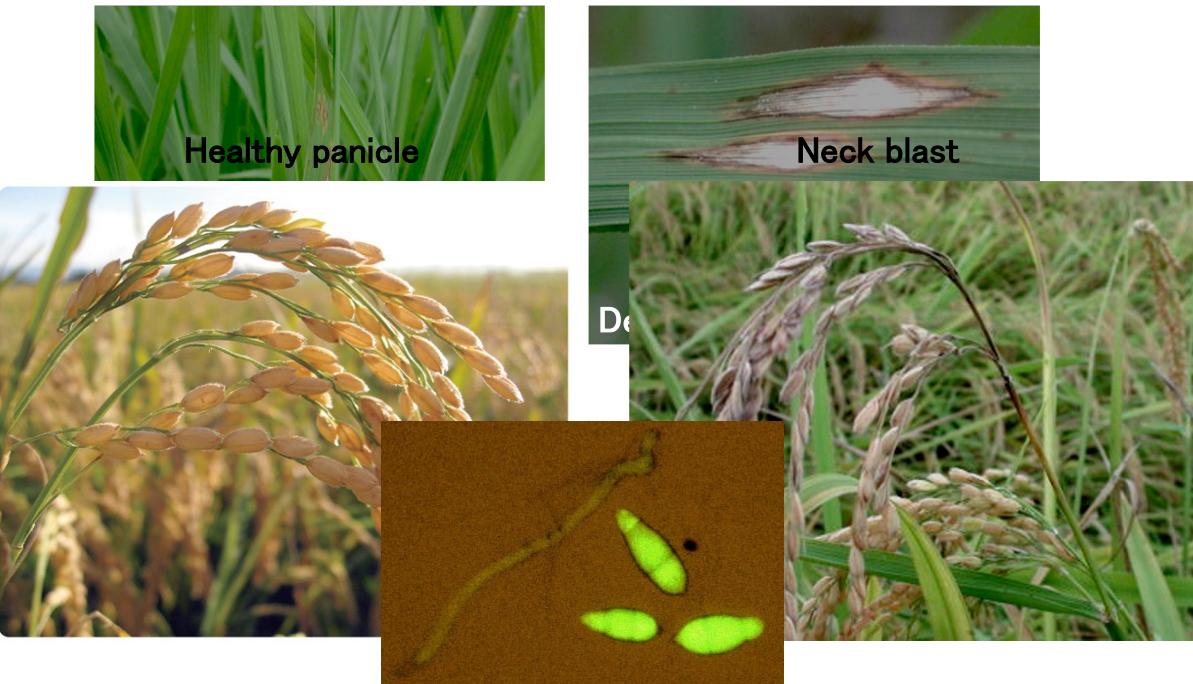


- By a cross to WT, MutMap allows isolation of gene affecting subtle changes in phenotype



## Rice blast disease caused by *Magnaporthe oryzae*

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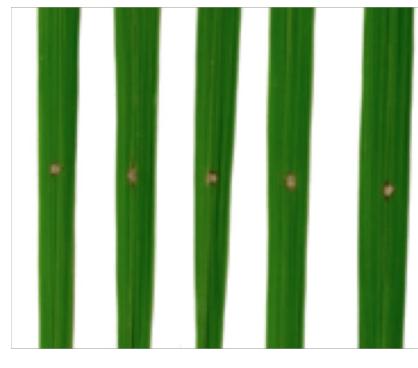


# MutMap identifies a blast resistance gene *Pii*

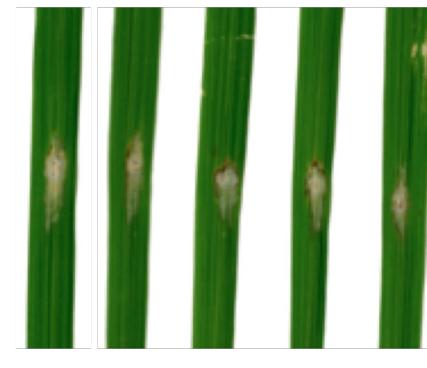
**Mutant : Hit 5948**

**Hitomebore (*Pii*) X Hit 5948**

↓  
**F<sub>2</sub>**



**WT**



**Mutant progeny**

**61**

**:**

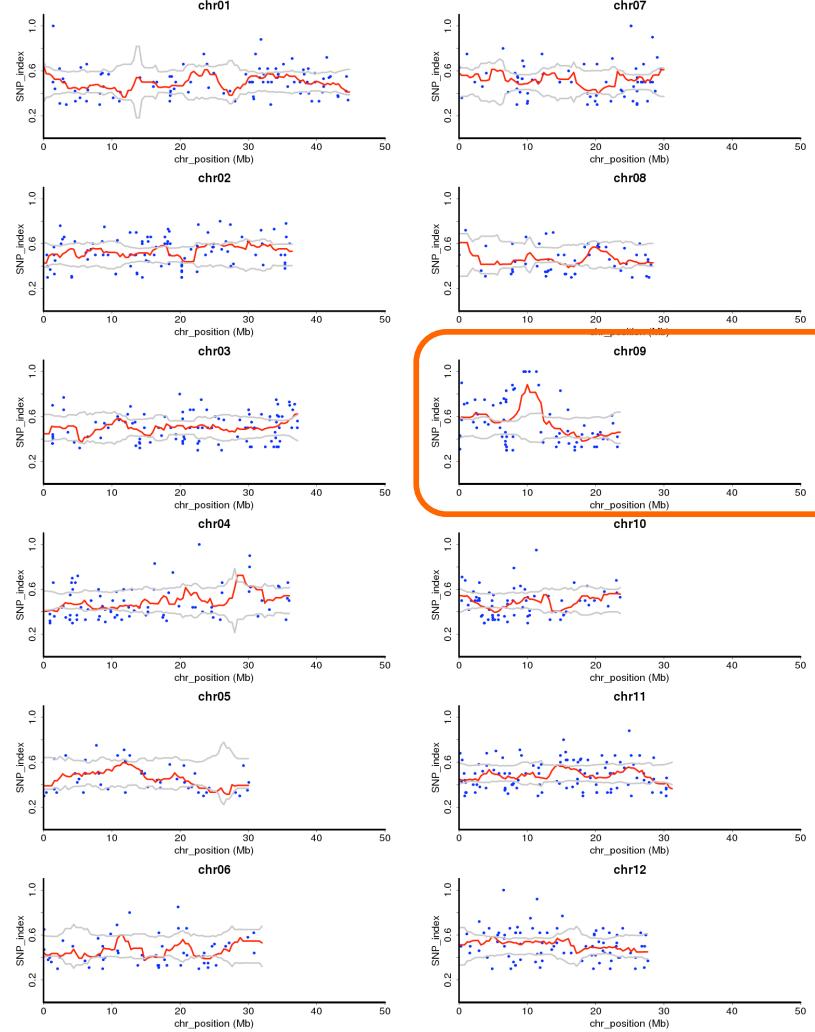
**17**

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

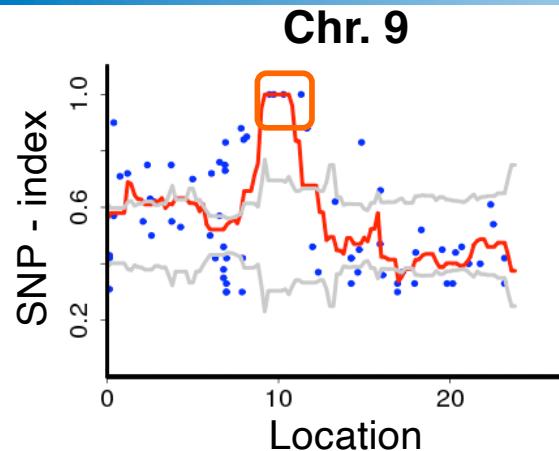
**MutMap**

# 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

MVGAEMLVAAVSQVARKINGIVGVAQGEVKLCCNFSDLEGIKDTLVY  
LETLLKNAENNSFGSDRANLRH WLQKI SLAYDIED DIVDGYSSKEQFD  
GGSYAQKGSLFCSLSNPMLLKGSMVYKMKSREMLQQRQLPNQYHFLS  
HINSAVNFEEKQTTSYRNTDIAIVGRDADLDNLMDILMENSAEELSIIS  
IVGPVGF GKTSLAQFVNNTGTEVFSFRI **W** VH VSMGNINLEKIGRD  
IVSQTTEKIEGNMQLQSIKNAVQRVLNKYSCLIILDSLWGKDEEVNELK  
QMLLTGRHTESKIIVTTHSNKVAKLISTVPLYKLAALSEDDCLKIFSQR  
AMTGP GDPLFREYGE EIVRRCEGTPVANFLGSVVNAQRQRREIWQAAK  
DEEMWKIEEDYPQDKISPLFPSFKIIYYNMPHELRLCFVYCSIFPKGT  
IEKKKLIQQWIALDMIEC

## Mutant

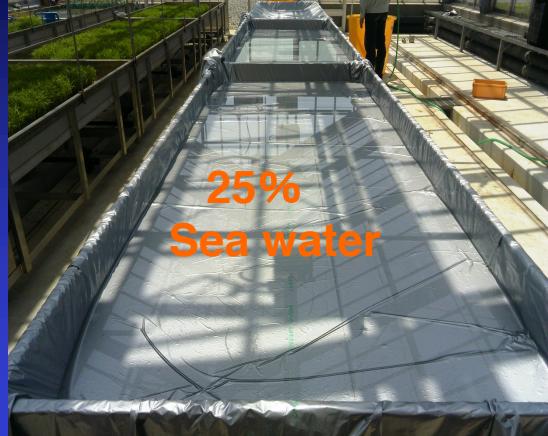
MVGAEMLVAAVSQVARKINGIVGVAQGEVKLCCNFSDLEGIKDTLVY  
LETLLKNAENNSFGSDRANLRH WLQKI SLAYDIED DIVDGYSSKEQFD  
GGSYAQKGSLFCSLSNPMLLKGSMVYKMKSREMLQQRQLPNQYHFLS  
HINSAVNFEEKQTTSYRNTDIAIVGRDADLDNLMDILMENSAEELSIIS  
→ IVGPVGF GKTSLAQFVNNTGTEVFSFRI **\*** VH VSMGNINLEKIGRD  
IVSQTTEKIEGNMQLQSIKNAVQRVLNKYSCLIILDSLWGKDEEVNELK  
QMLLTGRHTESKIIVTTHSNKVAKLISTVPLYKLAALSEDDCLKIFSQR  
AMTGP GDPLFREYGE EIVRRCEGTPVANFLGSVVNAQRQRREIWQAAK  
DEEMWKIEEDYPQDKISPLFPSFKIIYYNMPHELRLCFVYCSIFPKGT  
IEKKKLIQQWIALDMIEC

Takagi *et al.* New Phytol. 2013

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



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

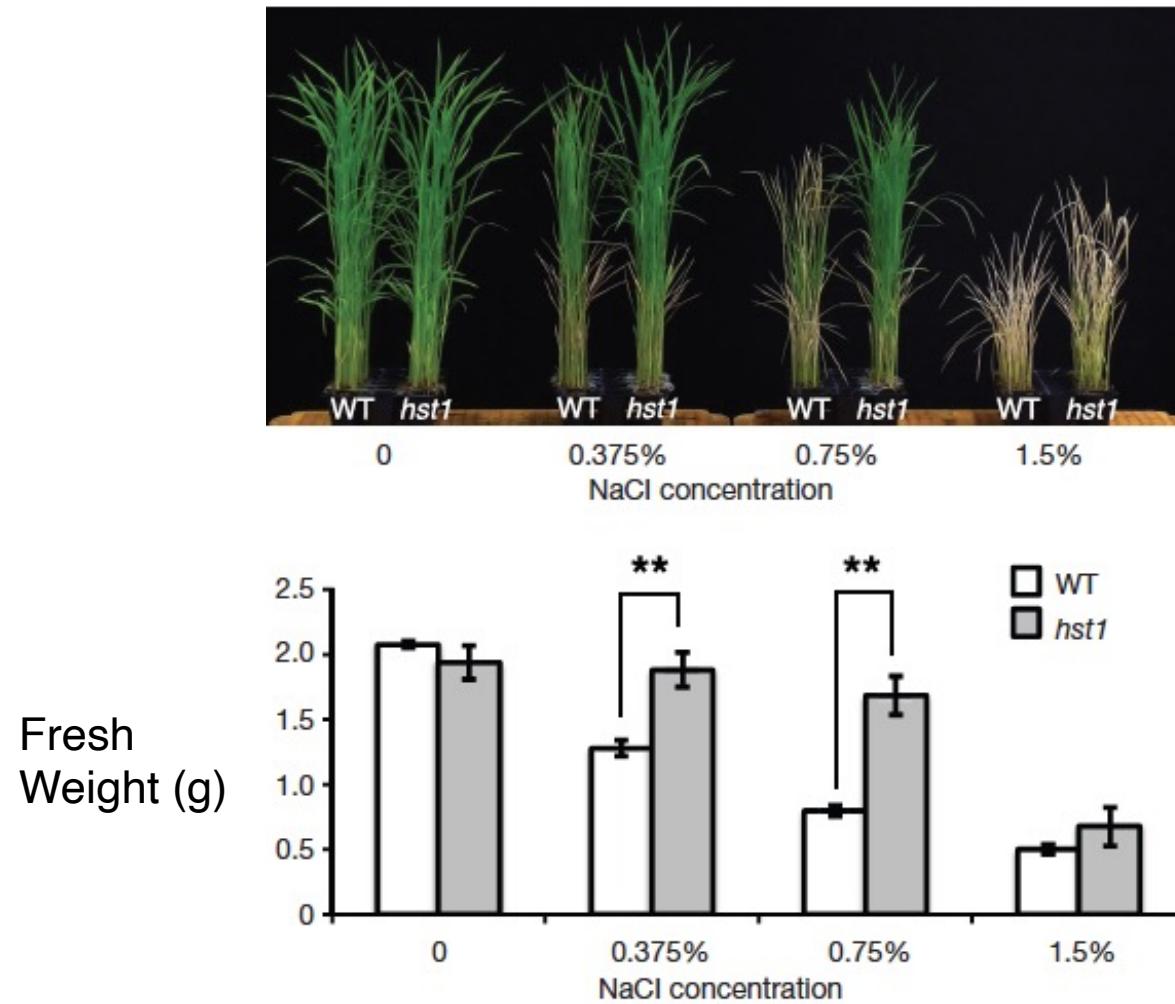


WT      *hst1*

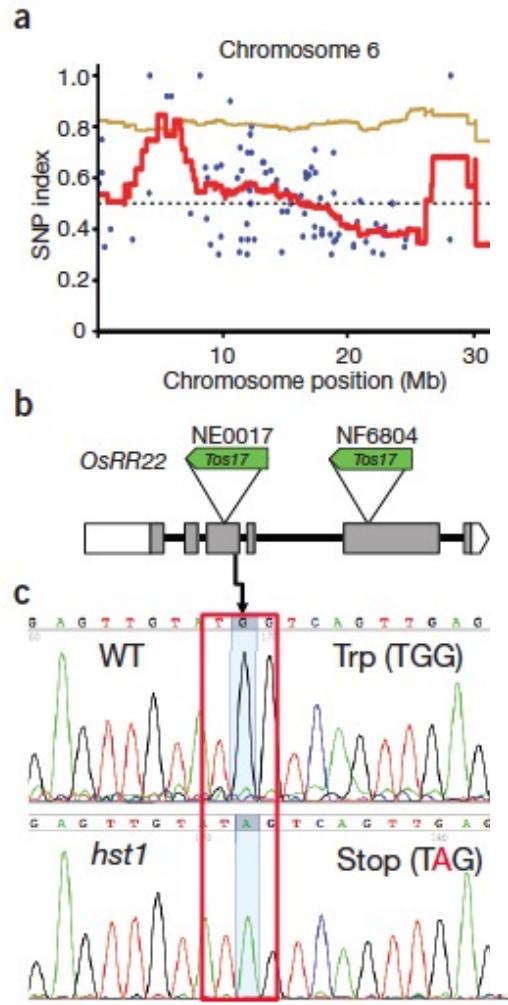
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0.75% NaCl (3 weeks)

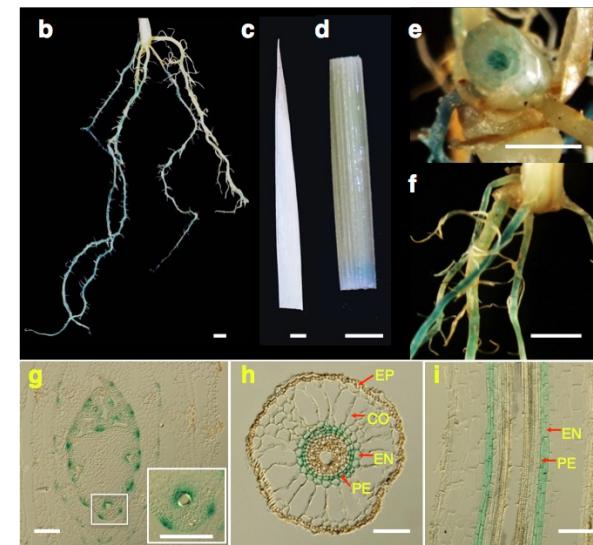
## Identification of *hst1* mutant with salinity tolerance



# MutMap identifies *OsRR22* gene responsible for salt tolerance



*OsRR22*:  
histidine kinase  
signaling pathway  
gene



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

## *hst1* shows salt tolerance in field

## Normal condition



## Salt-treated condition

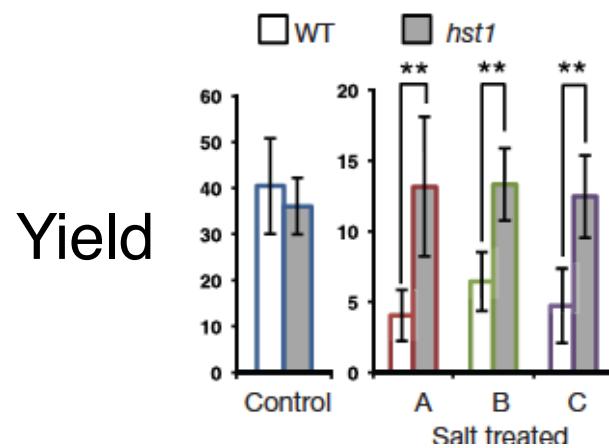


**Hitomebore**

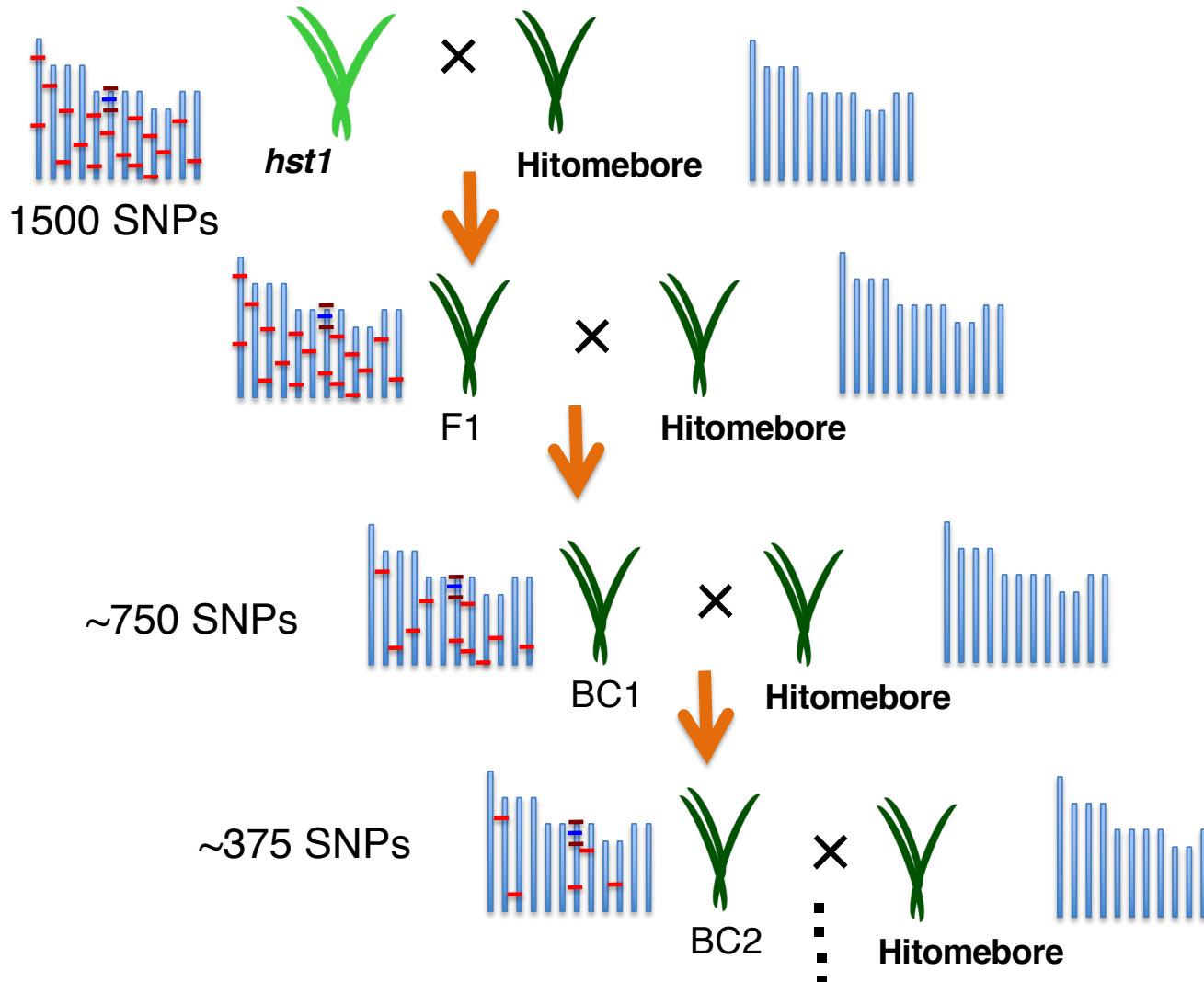
hst1

Hitomebore

hst1



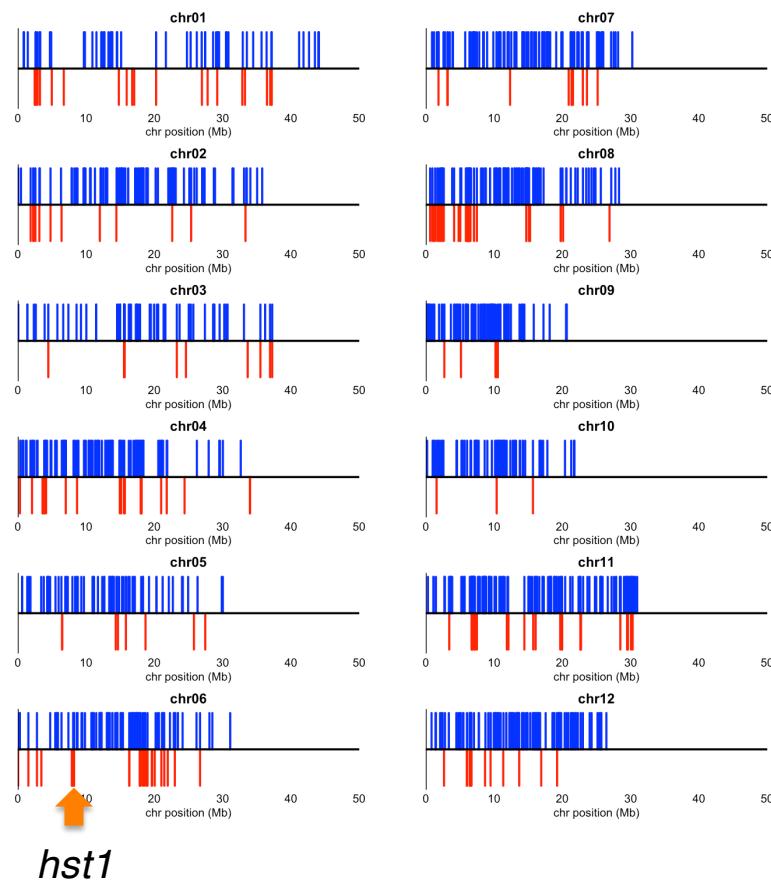
## Backcross of *hst1* to Hitomebore to reduce unnecessary mutations



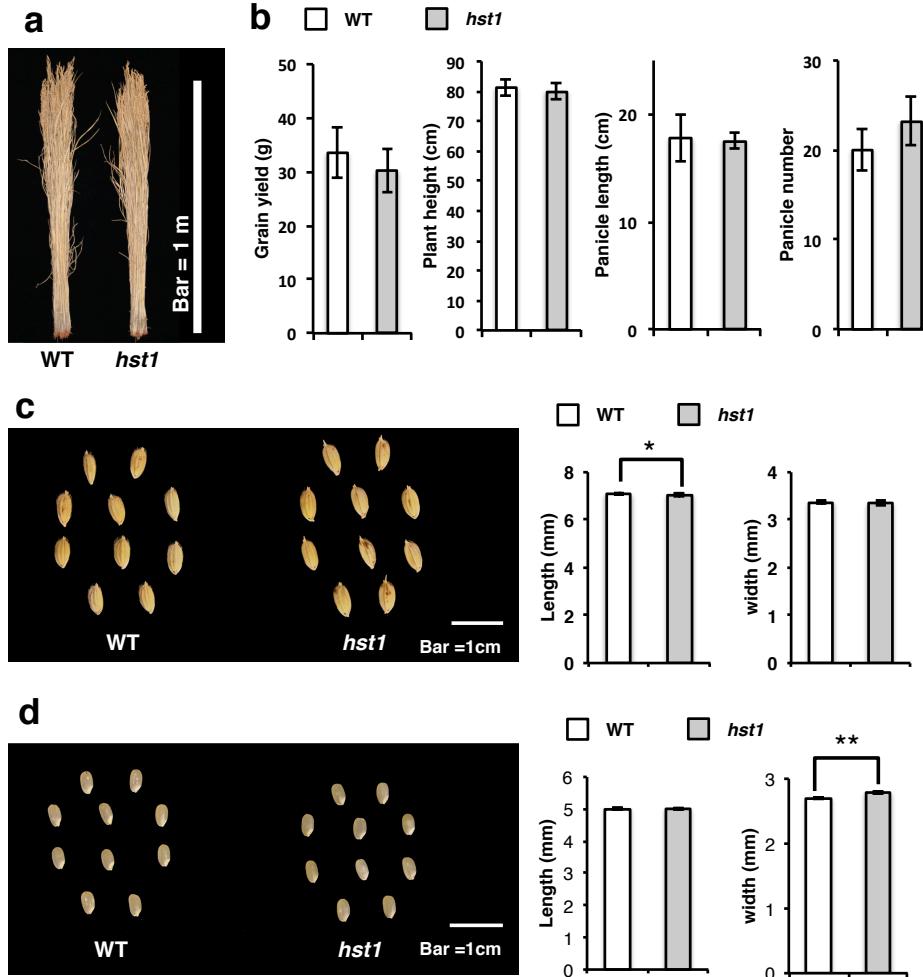
A variety “Kaijin” differs from Hitomebore only by 201

SNPs  
“Kaijin” in Japanese = Neptune

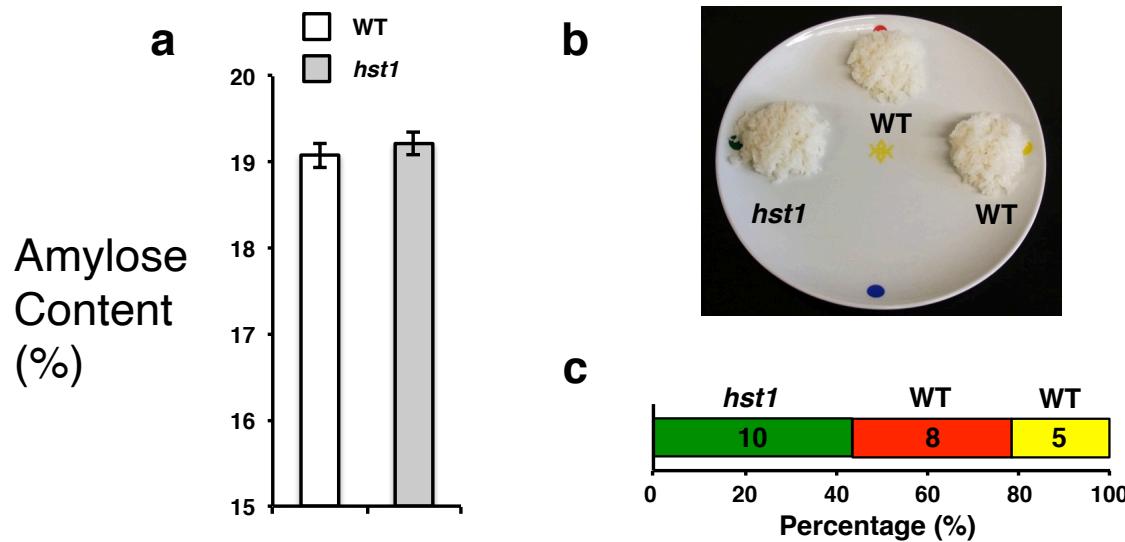
*hst1* (1088)  
Kaijin (201)



# 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.

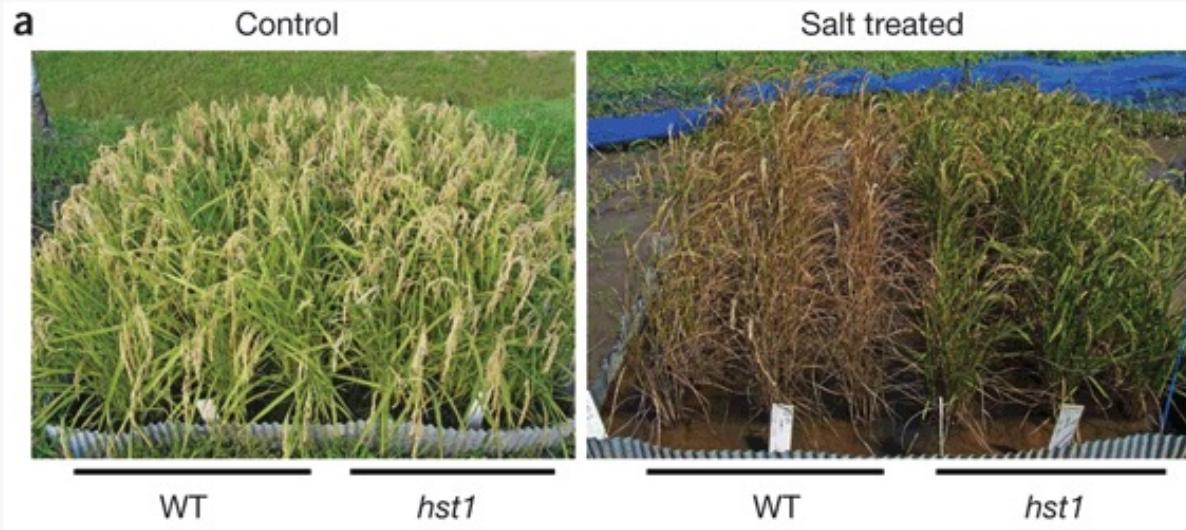
From

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



## High-performance pipeline for MutMap and QTL-seq

Yu Sugihara<sup>1,2</sup>, Lester Young<sup>3</sup>, Hiroki Yaegashi<sup>1</sup>, Satoshi Natsume<sup>1</sup>, Daniel J. Shea<sup>1</sup>, Hiroki Takagi<sup>4</sup>, Helen Booker<sup>3,5</sup>, Hideki Innan<sup>6</sup>, Ryohei Terauchi<sup>1,2</sup> and Akira Abe<sup>1</sup>

<sup>1</sup> Department of Genomics and Breeding, Iwate Biotechnology Research Center, Kitakami, Japan

<sup>2</sup> Graduate School of Agriculture, Kyoto University, Kyoto, Japan

<sup>3</sup> Department of Plant Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

<sup>4</sup> Faculty of Bioresources and Environmental Sciences, Ishikawa Prefectural University, Nonoichi, Japan

<sup>5</sup> Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada

<sup>6</sup> Graduate University for Advanced Studies, Hayama, Japan

### ABSTRACT

**Summary.** Bulked segregant analysis implemented in MutMap and QTL-seq is a powerful and efficient method to identify loci contributing to important phenotypic traits. However, the previous pipelines were not user-friendly to install and run. Here, we describe new pipelines for MutMap and QTL-seq. These updated pipelines are approximately 5–8 times faster than the previous pipeline, are easier for novice users to use, and can be easily installed through bioconda with all dependencies.

**Availability.** The new pipelines of MutMap and QTL-seq are written in Python and can be installed via bioconda. The source code and manuals are available online (MutMap: <https://github.com/YuSugihara/MutMap>, QTL-seq: <https://github.com/YuSugihara/QTL-seq>).

**Subjects** Bioinformatics, Computational Biology, Genetics, Genomics, Plant Science

**Keywords** Bioinformatics, Bulked-segregant analysis, QTL analysis, Mutation mapping, Agricultural science

## MutMap User Guide

version 2.3.4

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- [What is MutMap?](#)
- [Installation](#)
  - [Dependencies](#)
  - [Installation using bioconda](#)
  - [Manual installation](#)
- [Usage](#)
  - [Example 1 : run MutMap from FASTQ without trimming](#)
  - [Example 2 : run MutMap from FASTQ with trimming](#)
  - [Example 3 : run MutMap from BAM](#)
  - [Example 4 : run MutMap from multiple FASTQs and BAMs](#)
  - [Example 5 : run MutPlot from VCF](#)
- [Outputs](#)
- [About multiple testing correction](#)
- [Built and use your own database for snpEff](#)

Sugihara et al. 2022 Peer J

## Genetic resources

X

WGS

Powerful tools for isolation of  
important crop genes

MutMap/QTL-seq analyses pipelines  
publicly available: <http://www.ibrc.or>



Coworkers:

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

Welcome students/post-docs. Visit us at <http://www.ibrc.or.jp>

## Acknowledgements

### Collaborators:

Sophien Kamoun (Sainsbury Lab., UK)

Hideo Matsumura (Shinshu University, Japan)

Naoya Urasaki (Okinawa Agricultural Res. Center)

Innan Hideki (SOKENDAI, Japan)

### Fundings:

PROBRAIN, Japan

MAFF, Japan

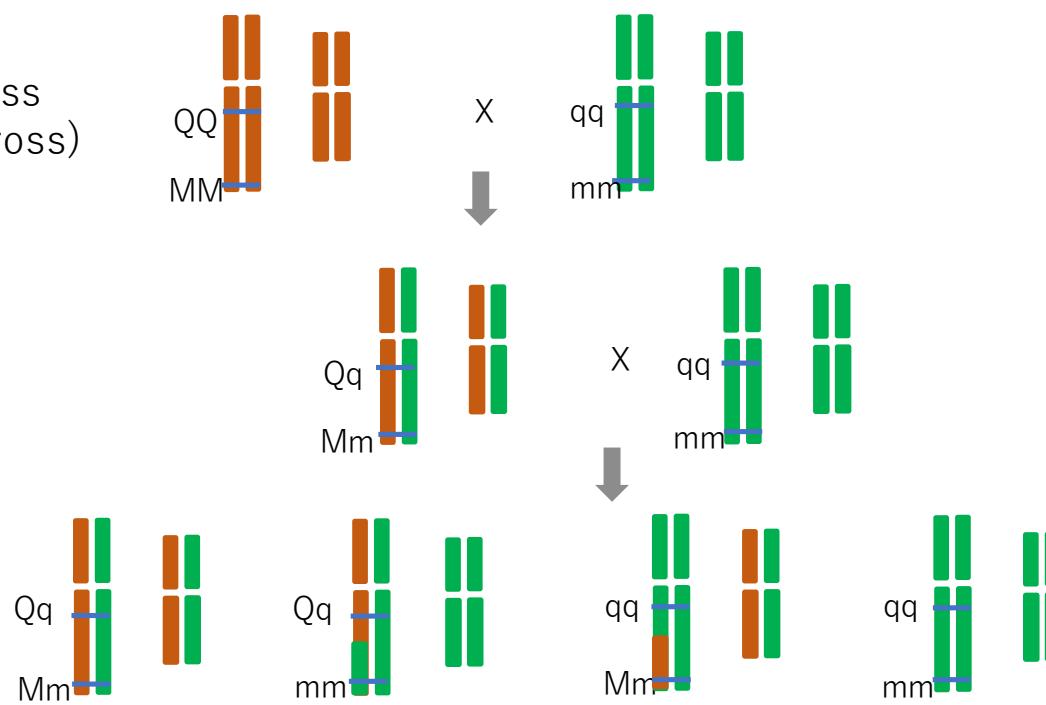
MEXT, Japan

JSPS, Japan

**QTL-seq**

## QTL mapping

Test cross  
(Back cross)



## Simple ANOVA for QTL detection

QTL MAPPING & QUANTITATIVE RESISTANCE 483

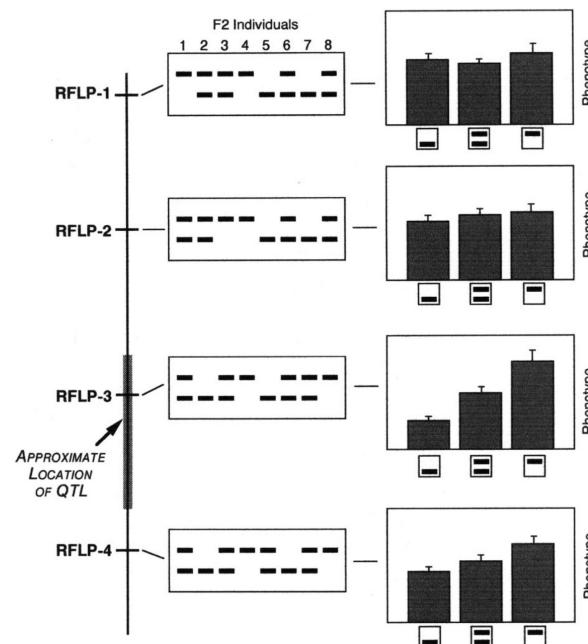


Figure 1 Conceptual basis of QTL mapping in an  $F_2$  population. DNA markers throughout the

Young 1996  
QTL mapping and  
Quantitative disease resistance in plants.  
Annu. Rev. Phytopathol. 34:479

LOD: logarithm of odd

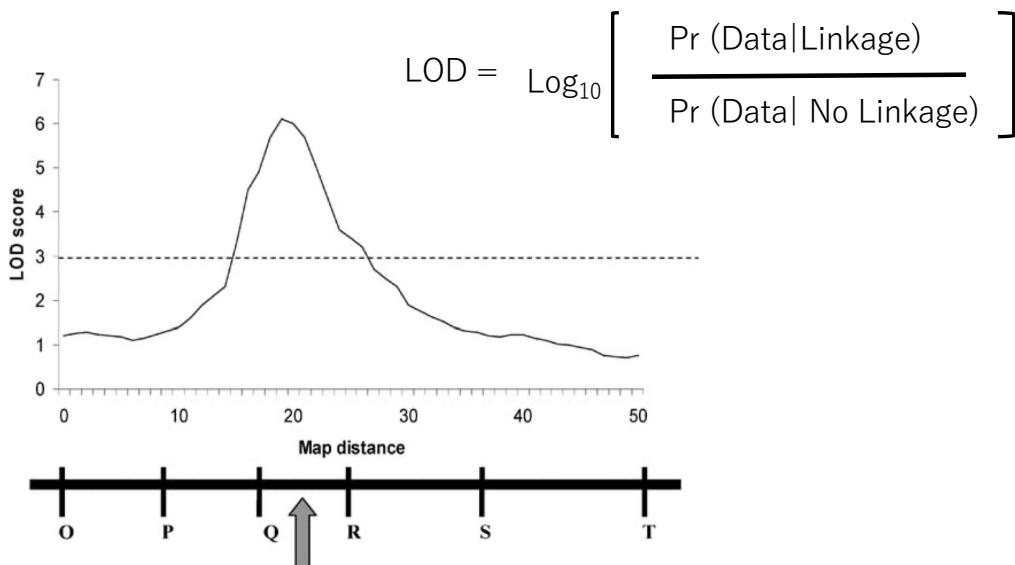


Figure 10. Hypothetical output showing a LOD profile for chromosome 4. The dotted line represents the significance threshold determined by permutation tests. The output indicates that the most likely position for the QTL is near marker Q (indicated by an arrow). The best flanking markers for this QTL would be Q and R.

Collard et al. 2005 Euphytica 142:169

## Bulked Segregant Analysis (BSA)

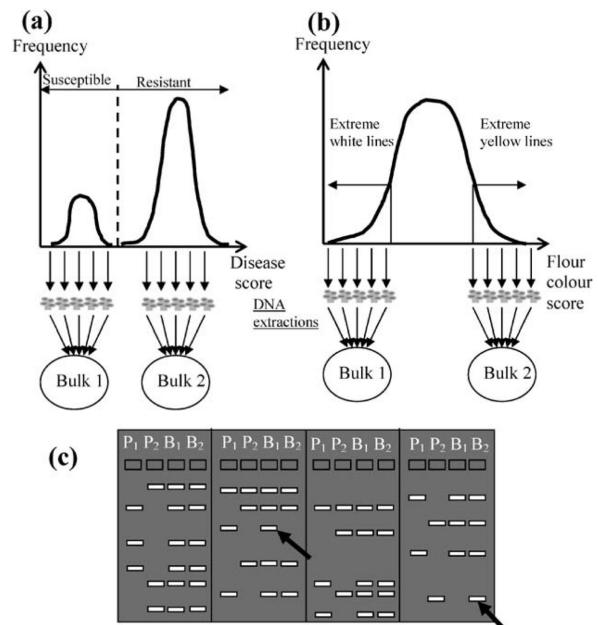


Figure 12. The preparation of DNA bulks for a simple disease resistance trait (a) and a quantitative quality trait (flower colour) (b). In both cases, two bulks ( $B_1$  and  $B_2$ ) are made from individuals displaying extreme phenotypic scores. (c) Polymorphic markers (indicated by arrows) that are identified between bulks may represent markers that are linked to genes or QTLs controlling the traits. Such markers are then used to genotype the entire mapping population and QTL analysis performed. (Adapted from Langridge et al., 2001; Tanksley et al., 1995.)

Michelmore et al. 1991  
PNAS

Collard et al. 2005 Euphytica 142:169

# QTL-seq

*the plant journal*



*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<sup>1,2</sup>, Akira Miyazaki<sup>3</sup>, Kentaro Yoshida<sup>4</sup>, Shunichi Kosegi<sup>5</sup>, Satoshi Natsume<sup>6</sup>, Chikako Mitsuoka<sup>7</sup>, Aiko Uemura<sup>8</sup>, Hiroe Ueda<sup>9</sup>, Mutsuo Tamura<sup>3</sup>, Shigenobu Takami<sup>9</sup>, Hitoshi Imanai<sup>10</sup>, Liliana M. Cano<sup>6</sup>, Sophieien Kamoun<sup>8</sup> and Ryohei Terauchi<sup>1,\*</sup>

<sup>1</sup>Iwate Biotechnology Research Center, Kitakami, Iwate 024-0003, Japan

<sup>2</sup>United Graduate School of Iwate University, Morioka, Iwate 029-0950, Japan

<sup>3</sup>Iwate Agricultural Research Center, Kitakami, Iwate 024-0003, Japan

<sup>4</sup>Graduate University for Advanced Studies, Hayama, Japan, and

<sup>5</sup>The Sainsbury Laboratory, Norwich Research Park, Norwich, UK

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



Hiroki Takagi

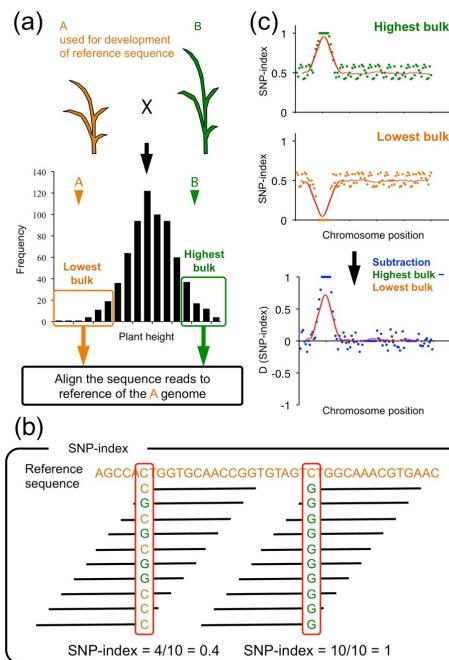
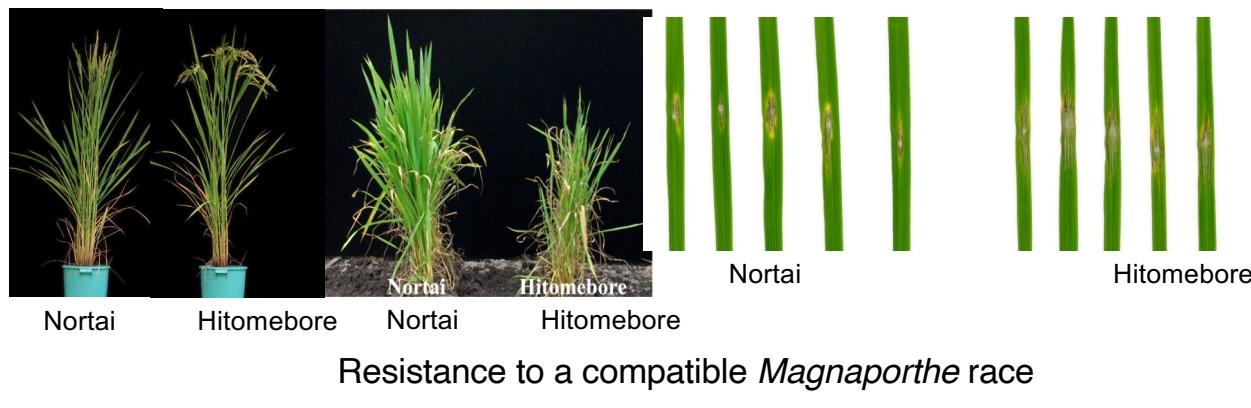


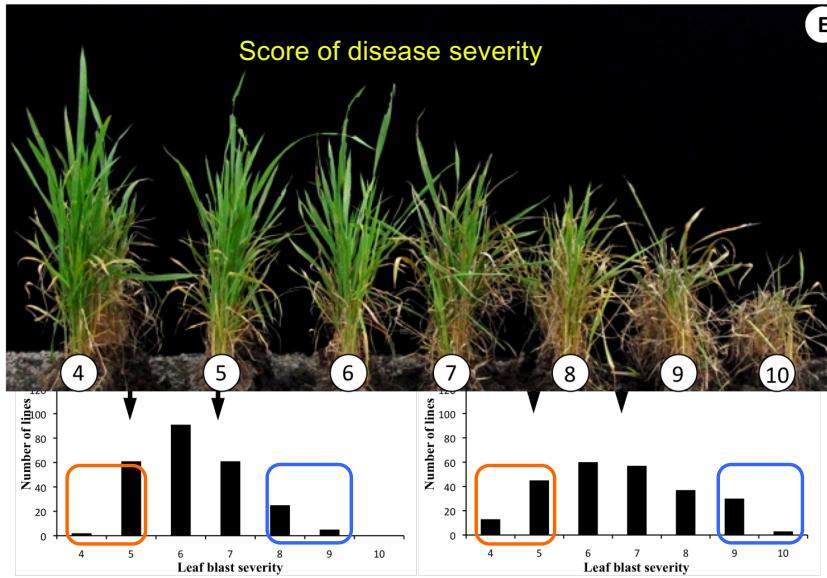
Figure 1.

QTL-seq:  
WGS and rapid mapping of QTL  
conferring rice field resistance



Nortai x Hitomebore      RIL (F7)    241 lines

E



## ”SNP-index” of NGS reads of bulked DNA

Hitomebore Wt = Reference

ACGCTCGATTTCTTCTTAATTAAATTGACACCAAGTTCCCTTTAGTGT  
\*  
TtGCATTTCTTCTTAATTAAATTG**g**C (+)  
CATTTCCTTCTTAATTAAATTG**g**CACC (-)  
CATTTCCTTCTTAATTAAATTG**g**CACC (+)  
TTCTTCTTAATTAAATTG**g**CACCAAG (+)  
TTCTTCTTAATTAAATTGACACCAAG (+)  
CTTCTTAATTAAATTGACACCAAGTT (+)  
TTCTTAATTAAATTGACACCAAGTTt (-)  
CTTAATTAAATTGACACCAAGTTc (-)  
CTTAATTAAATTGACACCAAGTTtCT (+)  
CTTAATTAAATTGACACCAAGTTtCT (+)

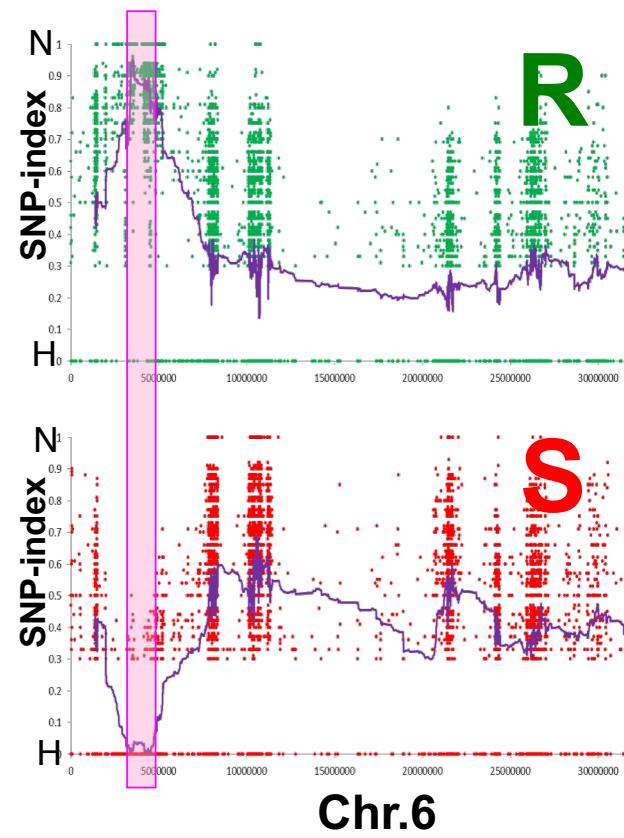
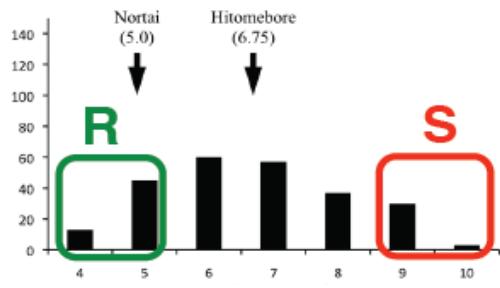
4 / 10 reads show “g”

SNP index = 0.4

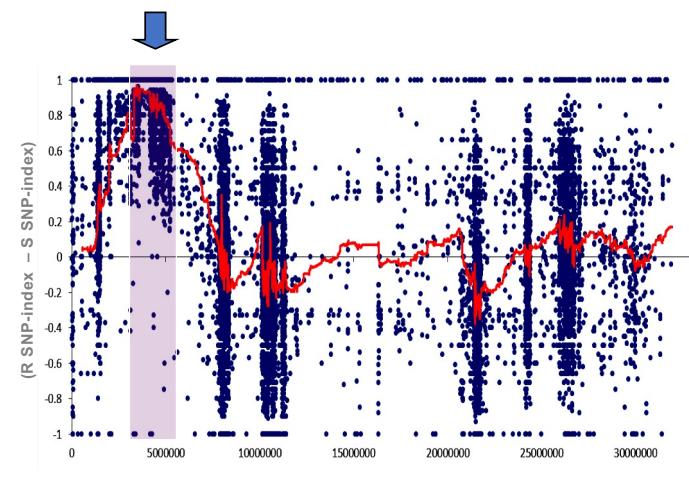
ACGCTCGATTTCTTCTTAATTAAATTGACACCAAGTTCCCTTTAGTGT  
\*  
TtGCATTTCTTCTTAATTAAATTG**g**C (+)  
CATTTCCTTCTTAATTAAATTG**g**CACC (-)  
CATTTCCTTCTTAATTAAATTG**g**CACC (+)  
TTCTTCTTAATTAAATTG**g**CACCAAG (+)  
TTCTTCTTAATTAAATTG**g**CACCAAG (+)  
CTTCTTAATTAAATTG**g**CACCAAGTT (+)  
TTCTTAATTAAATTG**g**CACCAAGTTt (-)  
CTTAATTAAATTG**g**CACCAAGTTc (-)  
CTTAATTAAATTG**g**CACCAAGTTtCT (+)  
CTTAATTAAATTG**g**CACCAAGTTtCT (+)

10 / 10 reads show “g”

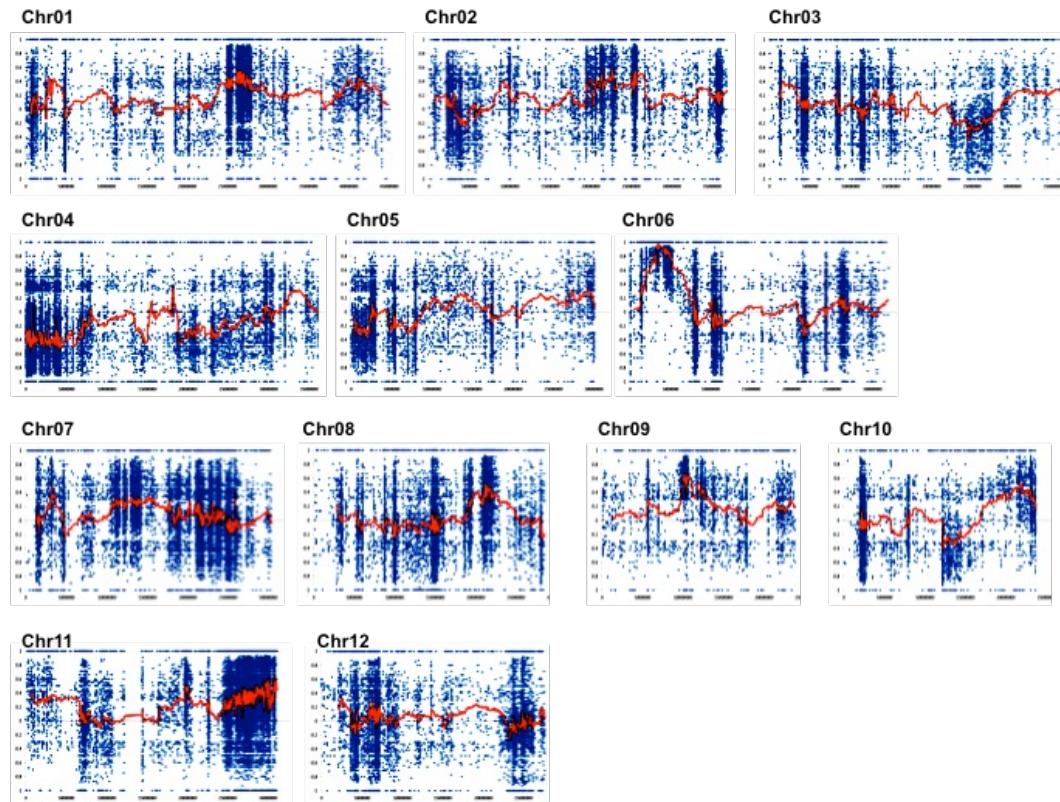
SNP index = 1.0



Plot of  $\Delta$ SNP-index  
= “SNP-index (R) – SNP-index (S)”

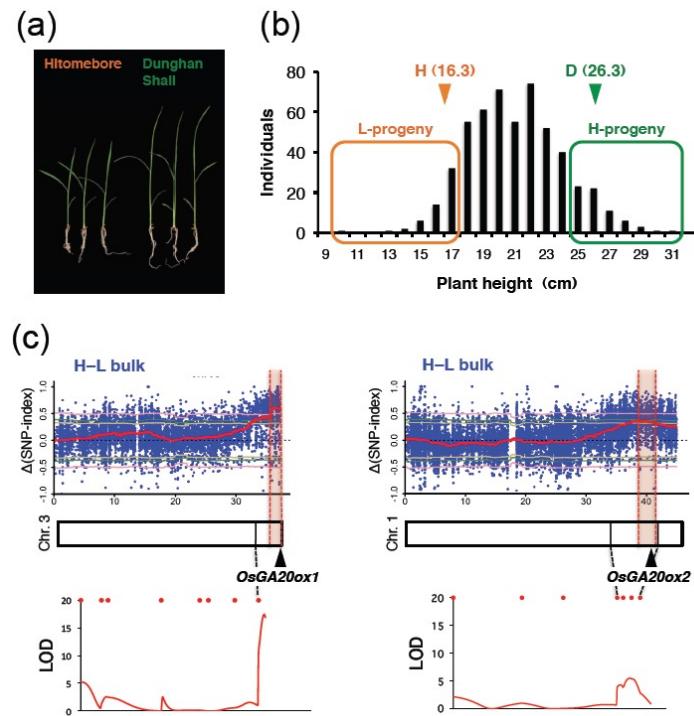


Chr. 6



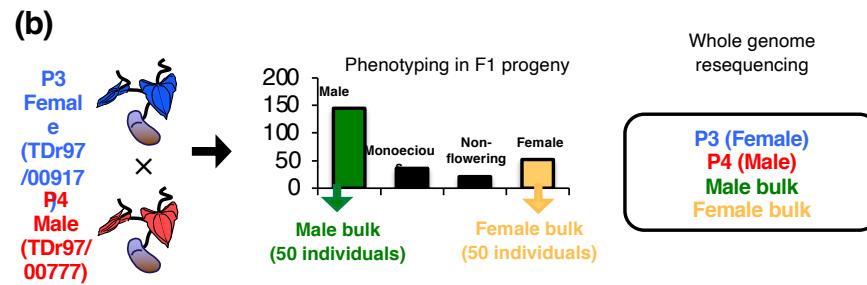
QTL-seq quickly identifies genomic regions under selective sweep

## Application of QTL-seq to F2

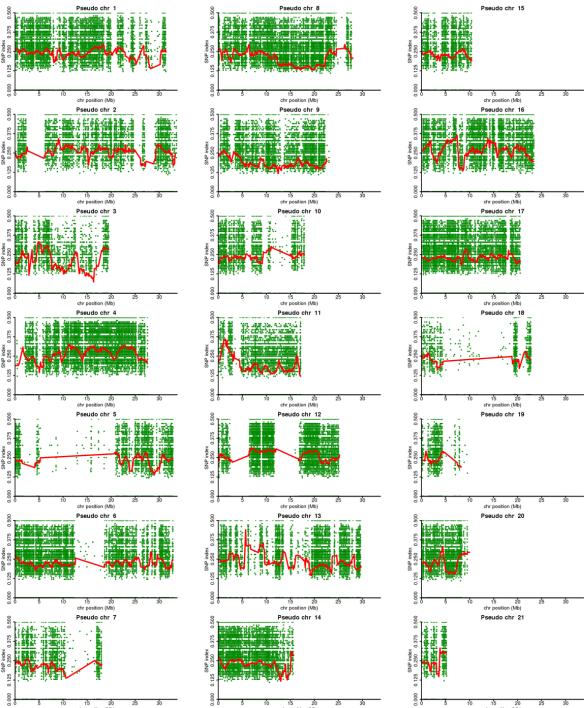


Takagi et al.  
*Plant J.* 74:174  
2013

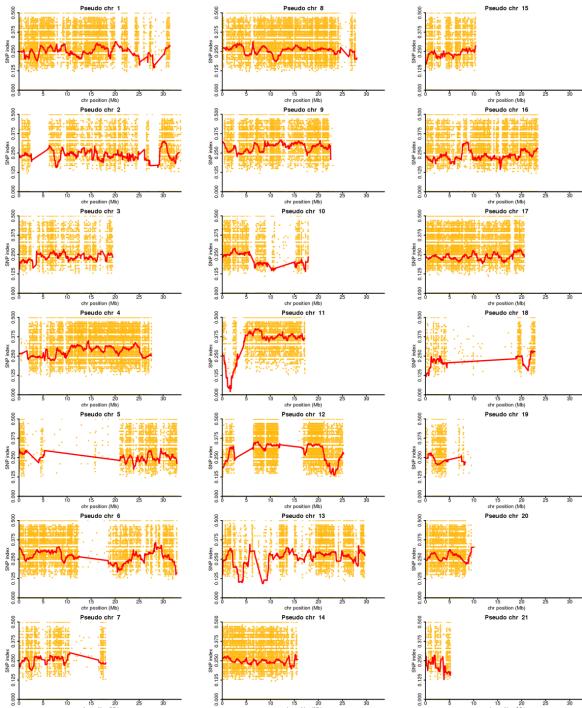
## Principles of QTL-seq as applied to F1 progeny of the highly heterozygous *D. rotundata*



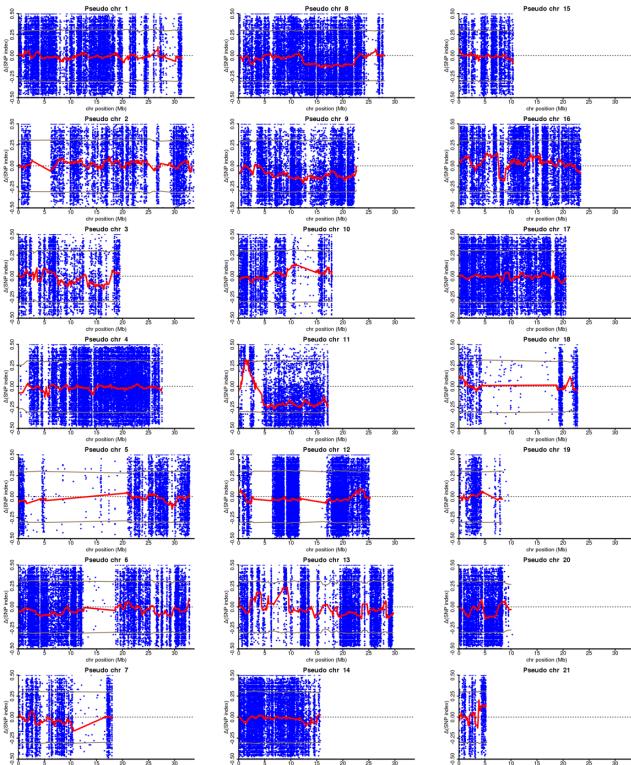
**(Male-bulk)**

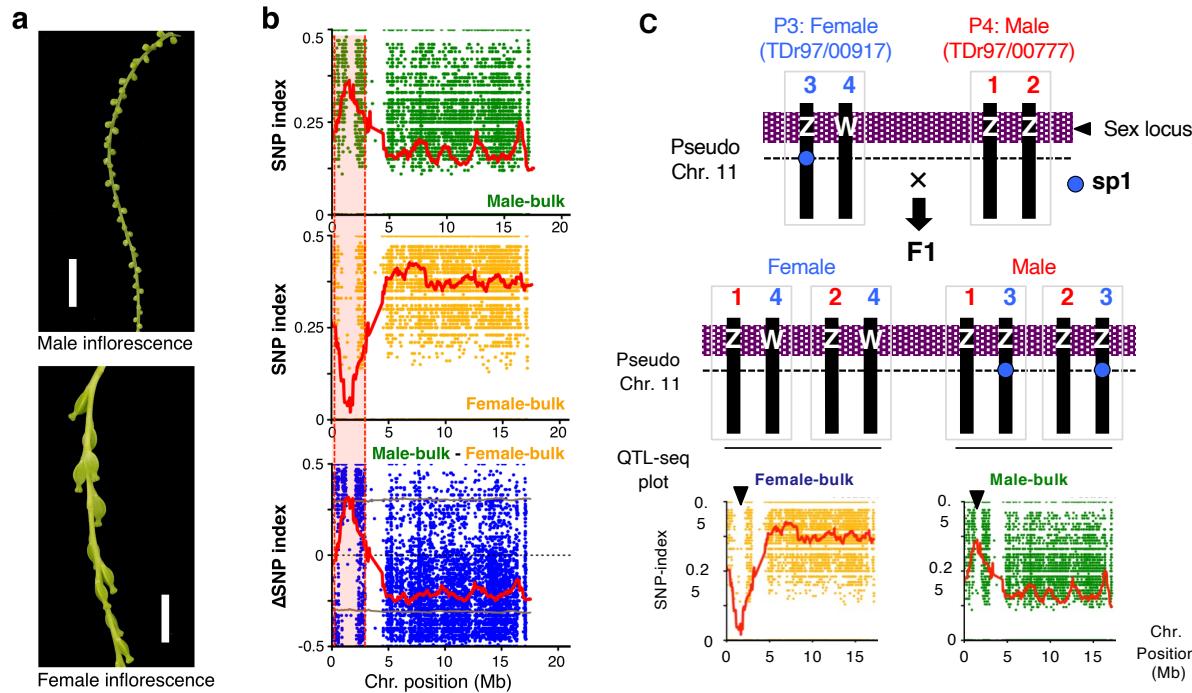


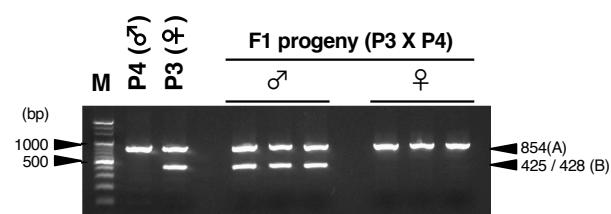
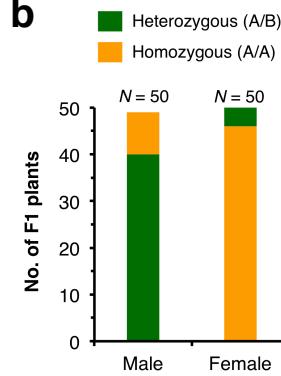
**(Female-bulk)**



$$\Delta\text{SNP-index} = (\text{Male-bulk}) - (\text{Female-bulk})$$

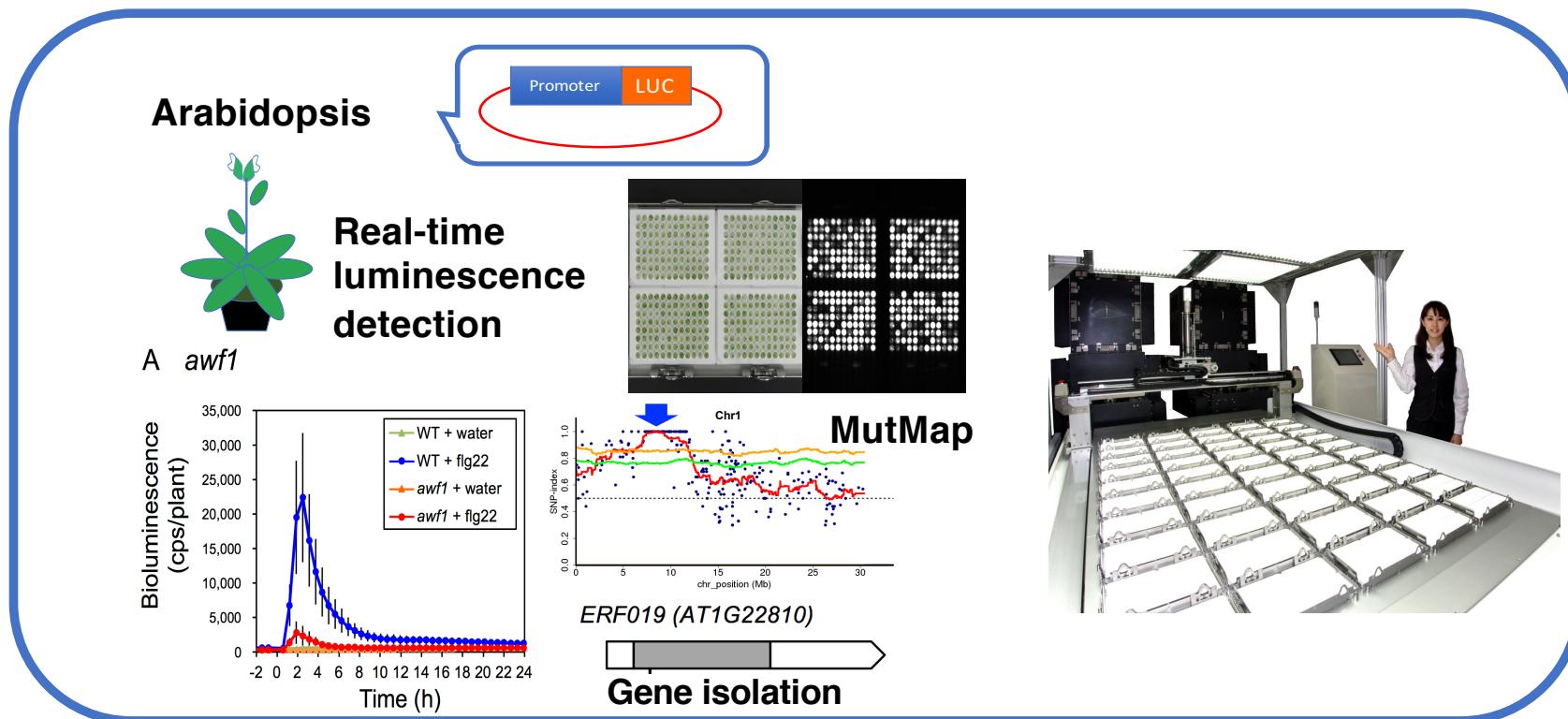




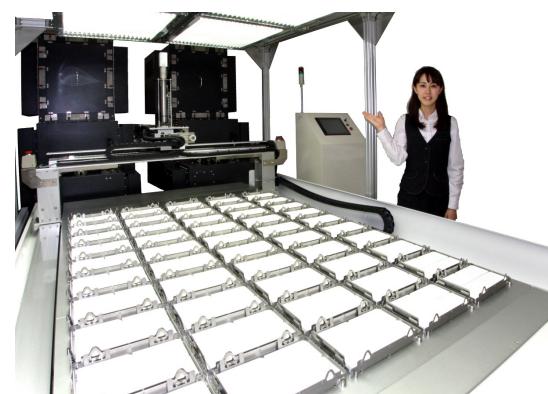
**a****b**



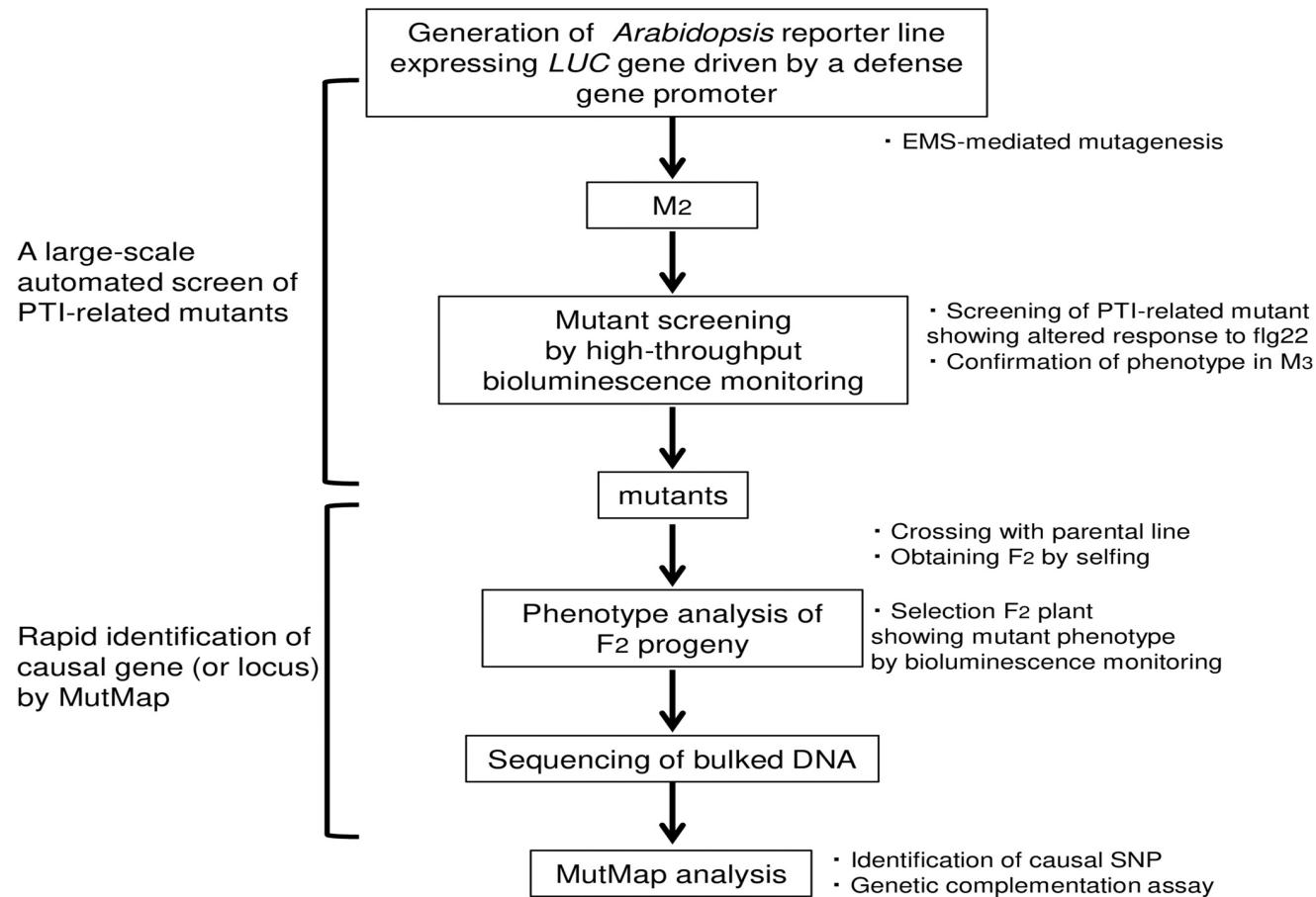
# Lumi-map



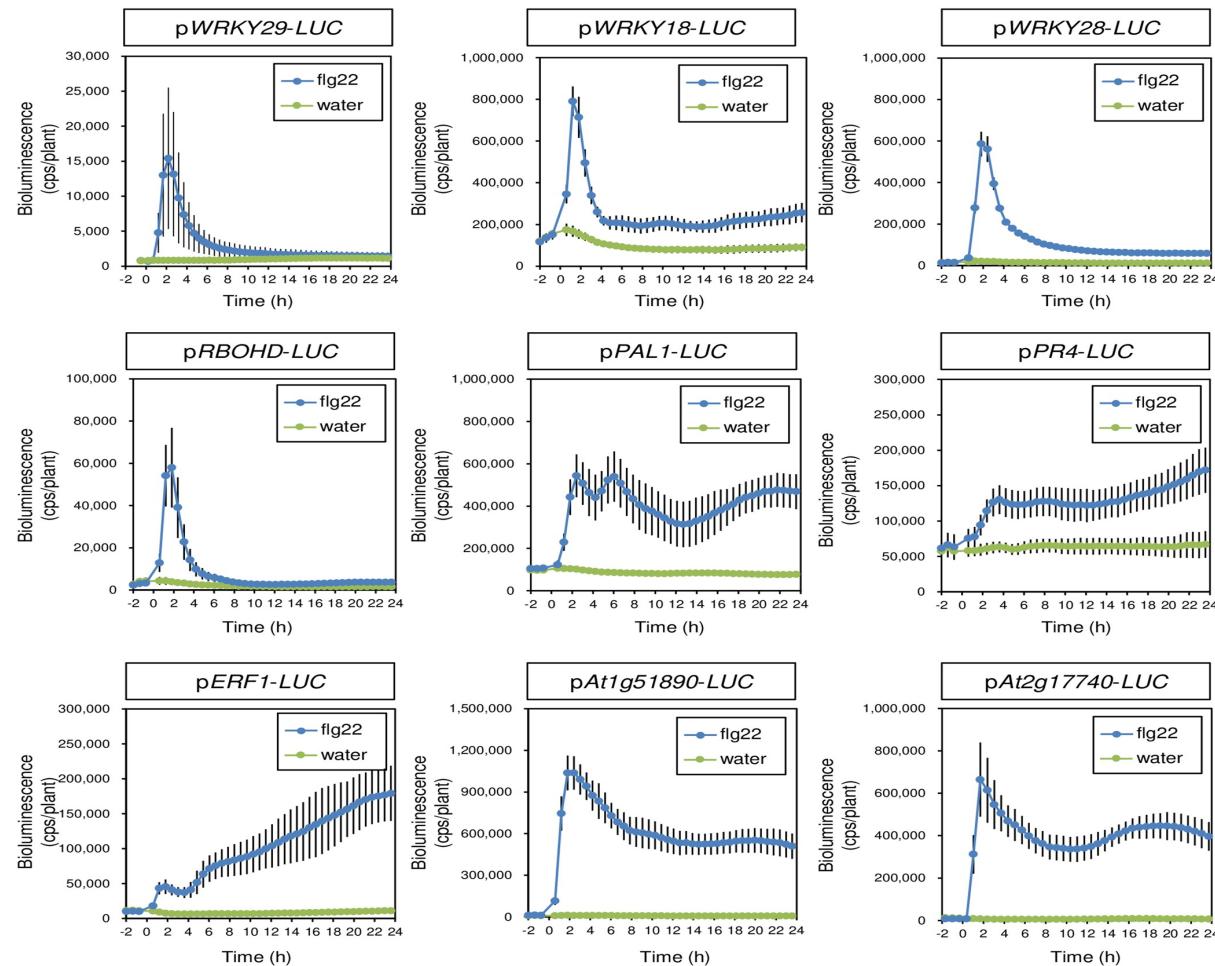
Hiroaki  
Kato



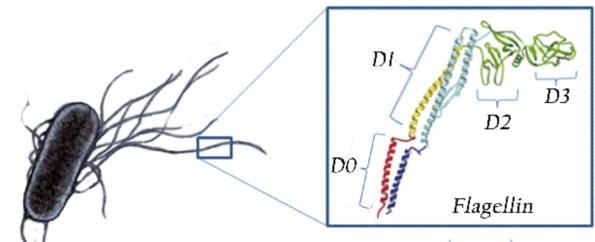
Kato et al. 2020 MPMI  
Kato et al. 2022 Science



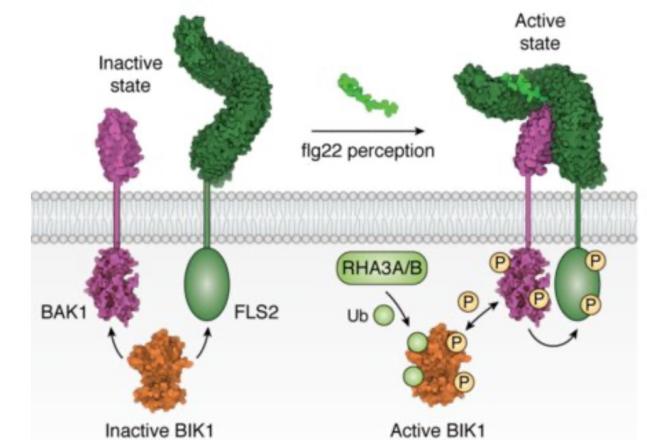
## Responses of different Arabidopsis promoters



## FLAG22 (flagellin)



<https://2008.igem.org/Team:Slovenia/Background/Flagellin>

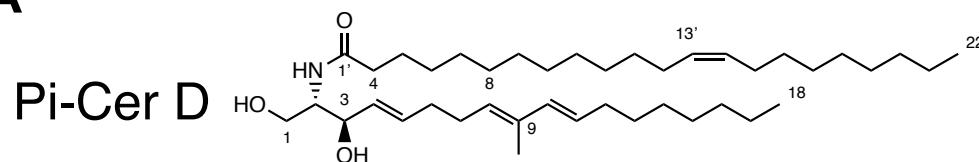


## FLG22 peptide

Bentham et al. JBC 2020

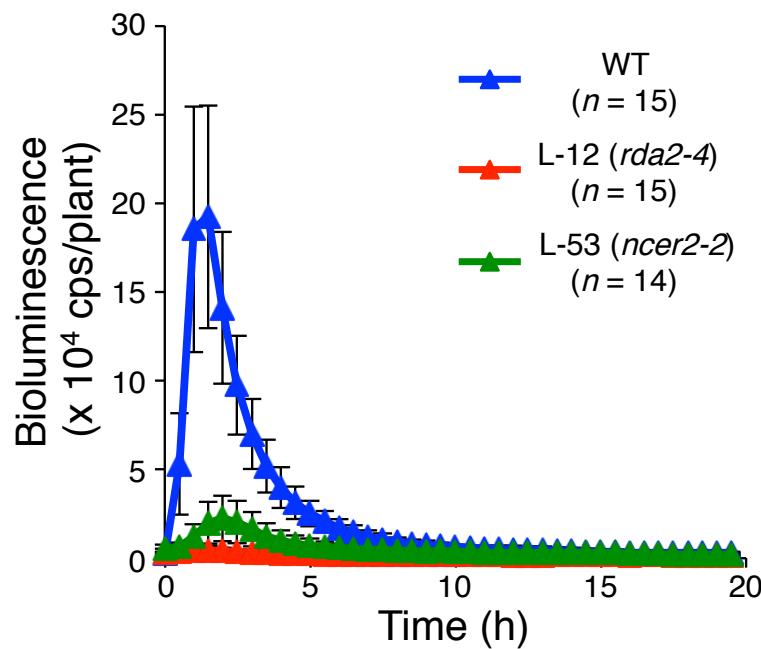
# Lipid Pi-Cer D activates WRKY33 promoter: No receptor known

A

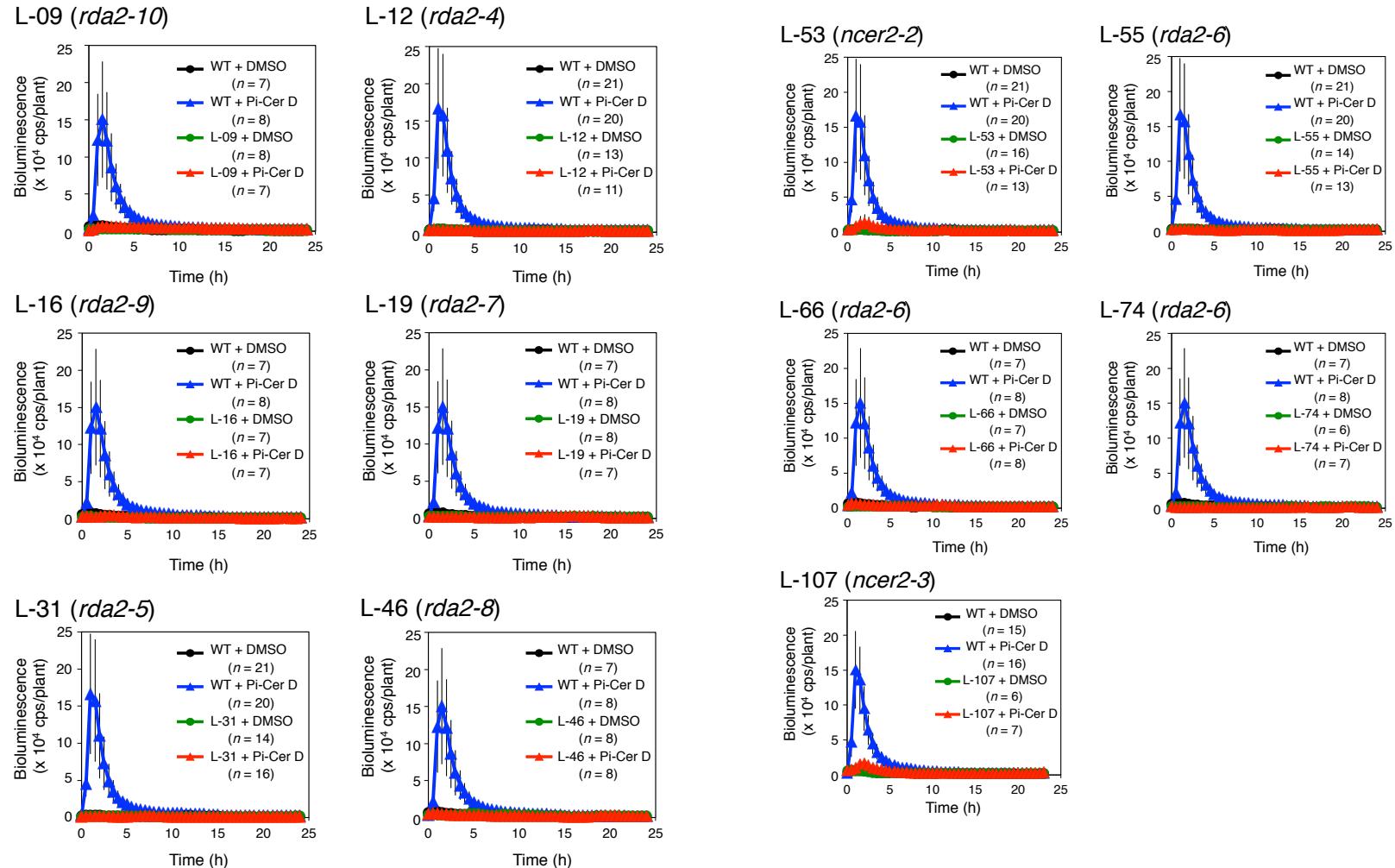


Ceramide D:  
component of  
oomycete and fungi

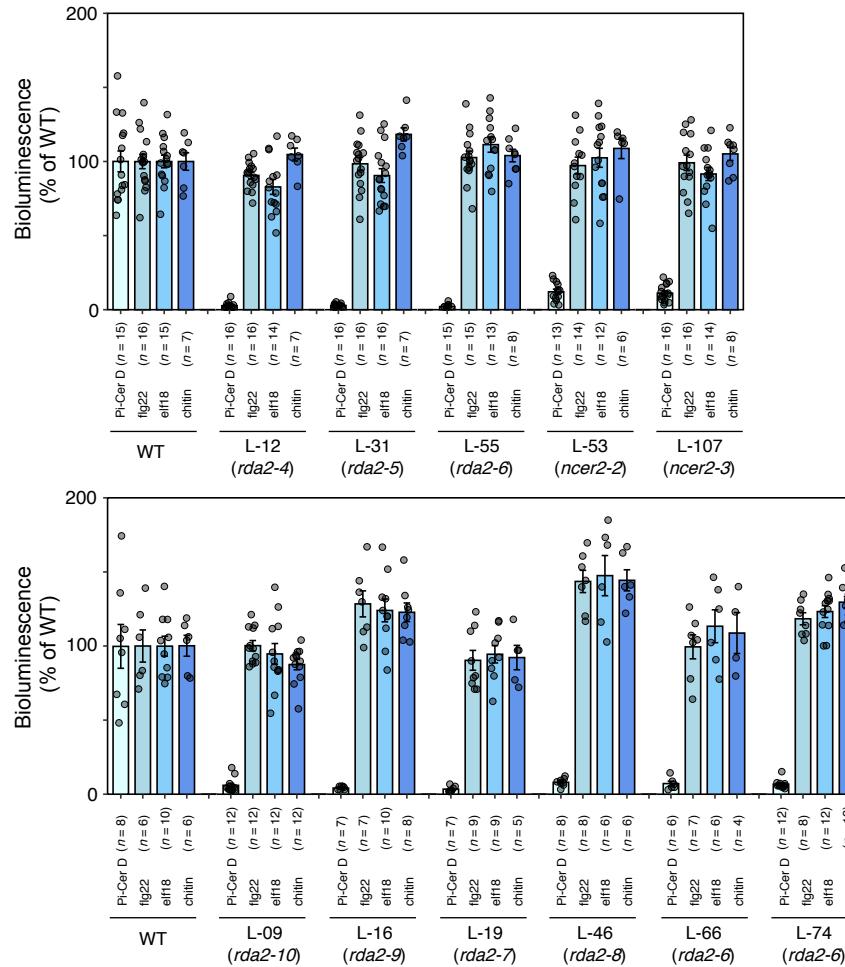
B



## Isolation of non-responsive mutants to PiCer-D

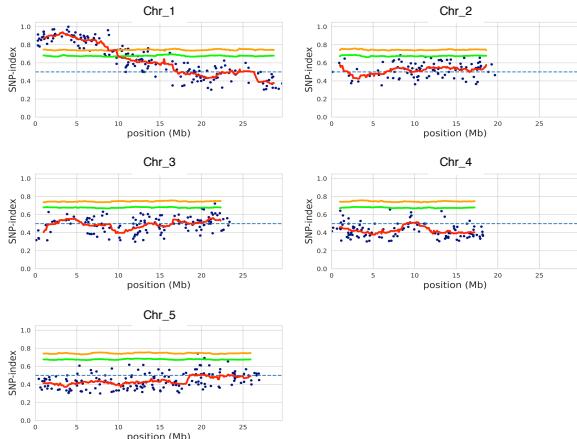


## Mutants are non-responsive only to PiCer-D

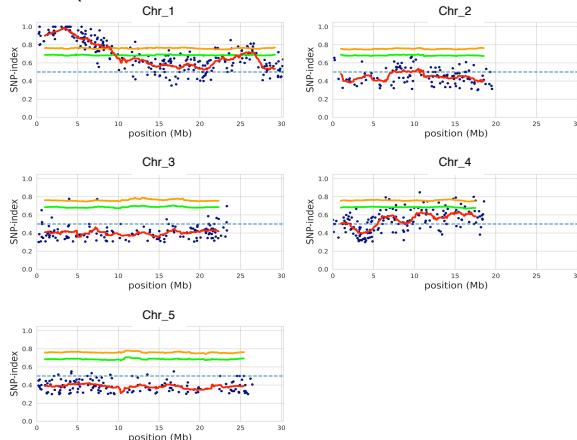


# MutMap of the mutants

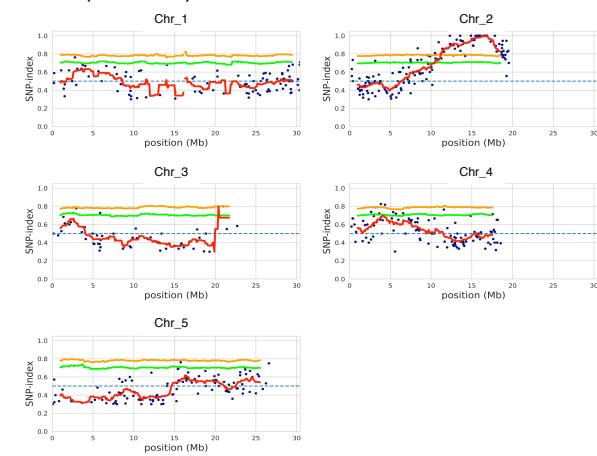
L-09 (*rda2-10*)



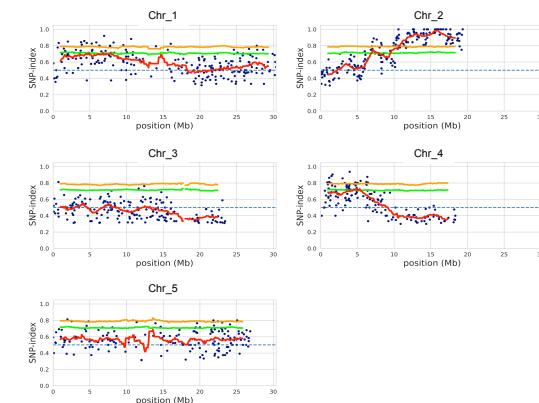
L-12 (*rda2-4*)



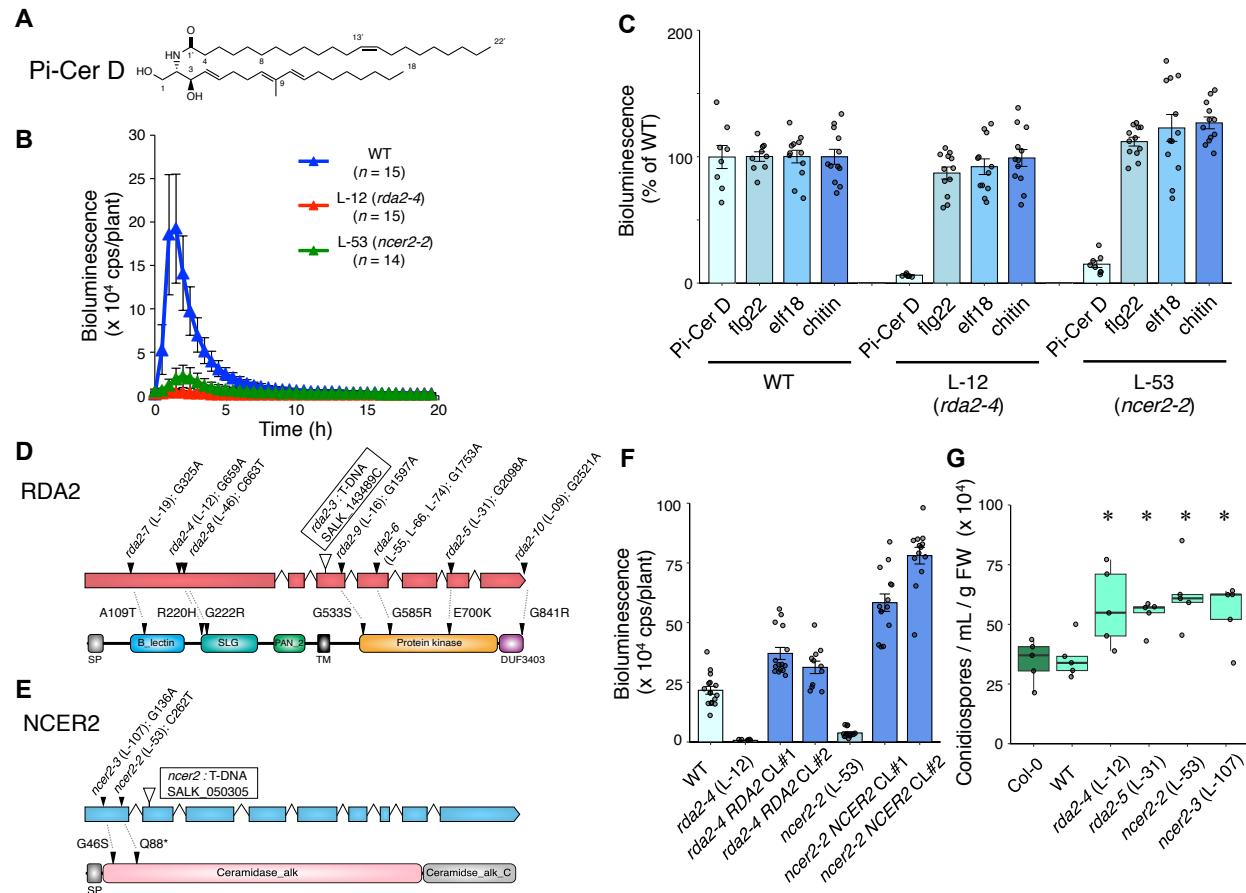
L-53 (*ncer2-2*)



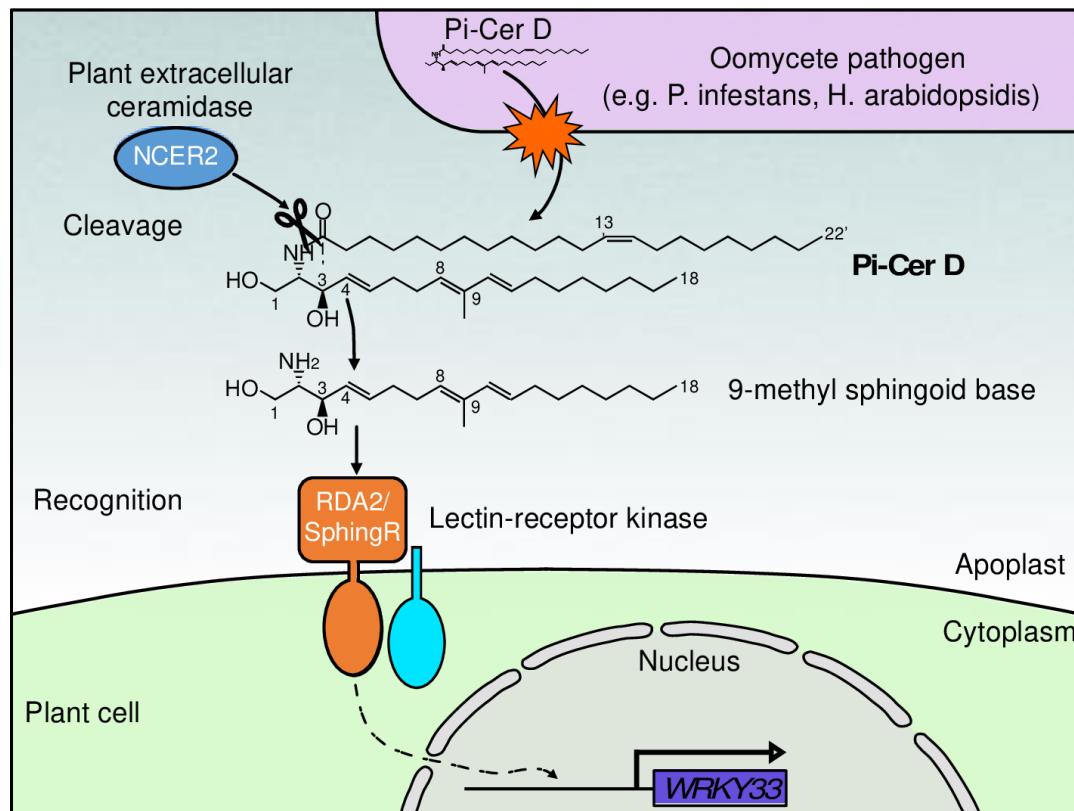
L-107 (*ncer2-3*)



# Lipid Pi-Cer D activates WRKY33 promoter: No receptor known



# Receptor identified: RDA2



## RESEARCH

### REPORT

#### PLANT SCIENCE

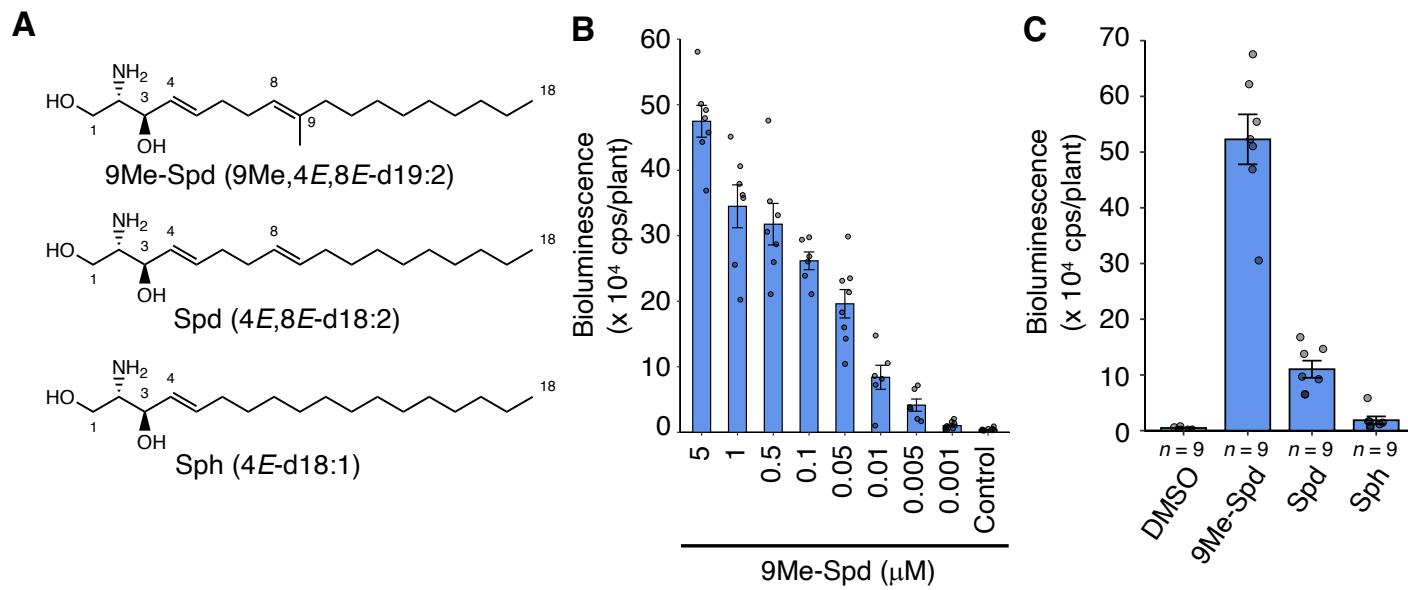
## Recognition of pathogen-derived sphingolipids in *Arabidopsis*

H. Kato<sup>1,\*</sup>, K. Nemoto<sup>2</sup>, M. Shimizu<sup>2</sup>, A. Abe<sup>2</sup>, S. Asai<sup>3</sup>, N. Itohama<sup>3</sup>, S. Matsuoka<sup>4</sup>, T. Daimon<sup>1</sup>, M. Ojika<sup>5</sup>, K. Kawakita<sup>5</sup>, K. Onai<sup>1</sup>, K. Shirasu<sup>3,6</sup>, M. Yoshida<sup>3,7</sup>, M. Ishiura<sup>8</sup>, D. Takemoto<sup>5</sup>, Y. Takano<sup>1</sup>, R. Terauchi<sup>1,2,\*</sup>

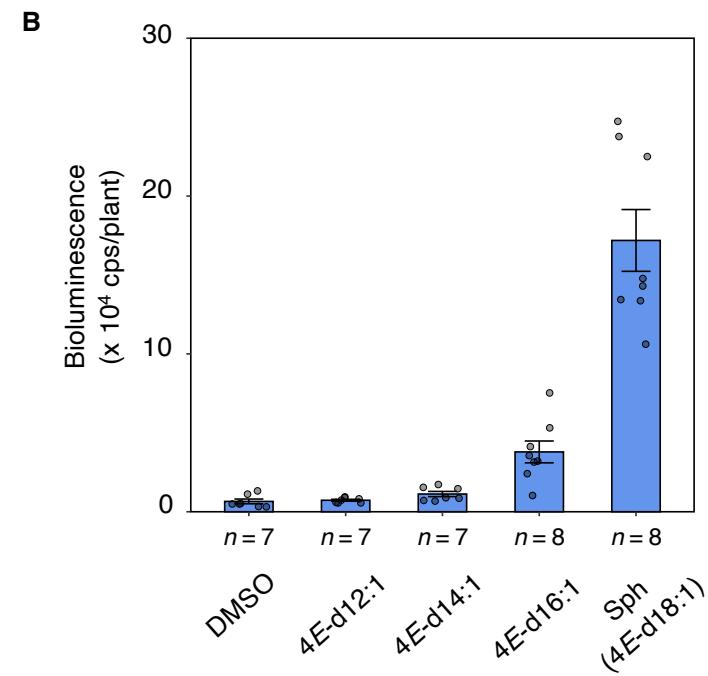
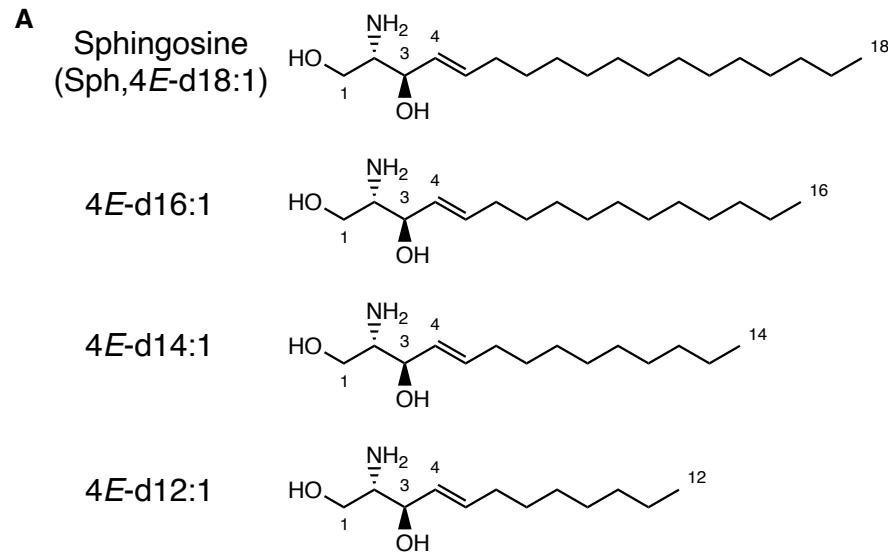
In plants, many invading microbial pathogens are recognized by cell-surface pattern recognition receptors (PRRs), which induce defense responses. Here, we show that the ceramide *Phytophthora infestans*-Ceramide D (Pi-Cer D) from the plant pathogenic oomycete *P. infestans* triggers defense responses in *Arabidopsis*. Pi-Cer D is cleaved by an *Arabidopsis* apoplastic ceramidase, NEUTRAL CERAMIDASE 2 (NCER2), and the resulting 9-methyl-branched sphingoid base is recognized by a plasma membrane lectin receptor-like kinase, RESISTANT TO DFPM-INHIBITION OF ABSCISIC ACID SIGNALING 2 (RDA2). 9-Methyl-branched sphingoid base is specific to microbes and induces plant immune responses by physically interacting with RDA2. Loss of *RDA2* or *NCER2* function compromised *Arabidopsis* resistance against an oomycete pathogen. Thus, we elucidated the recognition mechanisms of pathogen-derived lipid molecules in plants.

Kato et al. 2022 Science

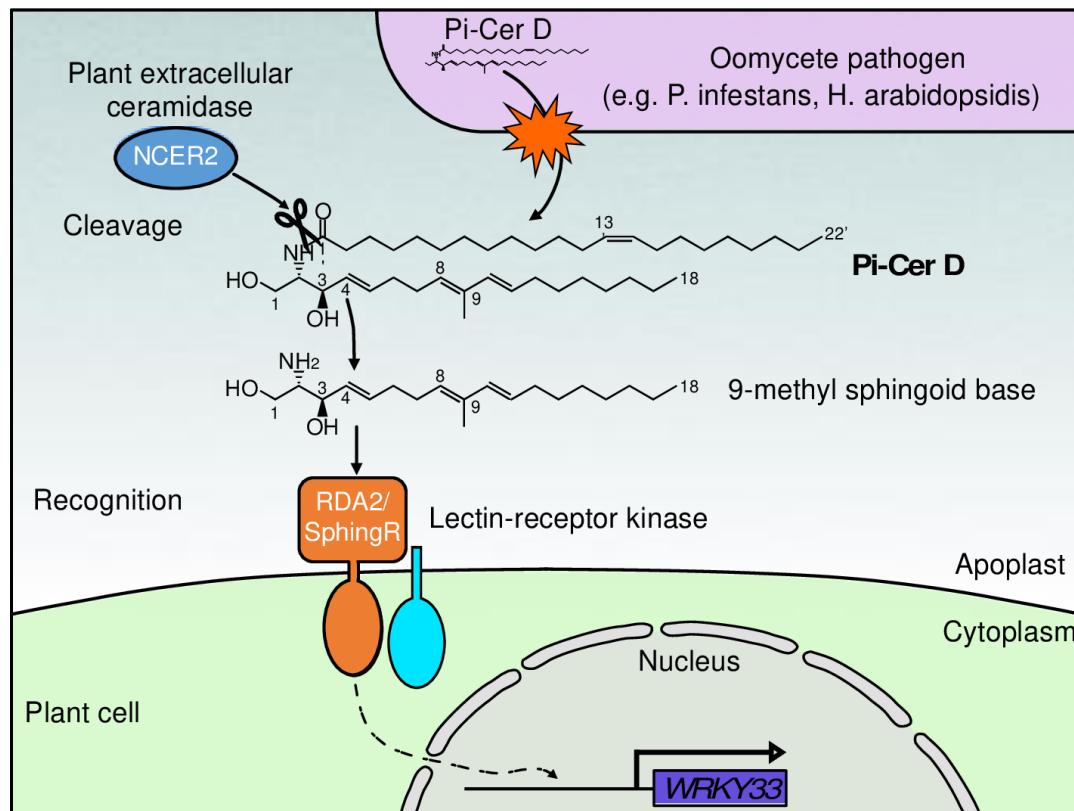
# RDA2 has specific recognition to 9Me-Spd



# RDA2 recognition affected by fatty acid chain length



# Receptor identified: RDA2



## RESEARCH

### REPORT

#### PLANT SCIENCE

## Recognition of pathogen-derived sphingolipids in *Arabidopsis*

H. Kato<sup>1,\*</sup>, K. Nemoto<sup>2</sup>, M. Shimizu<sup>2</sup>, A. Abe<sup>2</sup>, S. Asai<sup>3</sup>, N. Itohama<sup>3</sup>, S. Matsuoka<sup>4</sup>, T. Daimon<sup>1</sup>, M. Ojika<sup>5</sup>, K. Kawakita<sup>5</sup>, K. Onai<sup>1</sup>, K. Shirasu<sup>3,6</sup>, M. Yoshida<sup>3,7</sup>, M. Ishiura<sup>8</sup>, D. Takemoto<sup>5</sup>, Y. Takano<sup>1</sup>, R. Terauchi<sup>1,2,\*</sup>

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Kato et al. 2022 Science