

Bundesforschungsinstitut für Kulturpflanzen Federal Research Centre for Cultivated Plants

High-resolution mapping of the barley mild mosaic virus (BaMMV) resistance gene *rym15*

Yaping Wang, Antje Habekuß, Frank Ordon, Dragan Perovic

Institute for Resistance Research and Stress Tolerance

www.julius-kuehn.de

Introduction

Barley (Hordeum vulgare L.)

Diploid species: 2n=14 chromosomes

Genome size: 5.2 Gb

Used for animal feed, malting and human nutrition

Host for more than 50 different viruses

BaMMV (barley mild mosaic virus)

Family Potyviridae, genus Bymovirus

Filamentous particles (500-600nm; 250-300nm)

Soil-borne virus, transmitted by protist *Polymyxa graminis*

5-10°C, high soil moisture conditions

Germany, France, Great Britain, other western European countries, Japan, China and South Korea

50% yield losses

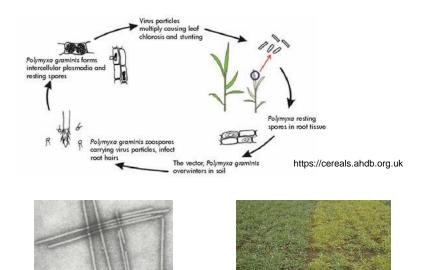






BaYMV https://pflanzenschutzdienst.rp-giessen.de

BaMMV





Purified virus particles, stained with sodium Resistant (left) and susceptible (right) cultivar on BaMMV- and BaYMV- infected field

Mapped Bymovirus resistance genes



	Resistance gene	Chromosome	Source	Resistance of donor	Virus used for mapping	ChxPI_6H	LixHs_6H
	rym1	4HL	Mokusekko 3	BaMMV, BaYMV, BaYMV-2	BaYMV ^b	EBmac08740.0	0.0 Bmag0173
	rym2	7HL	Mihori Hadaka 3	BaMMV, BaYMV, BaYMV-2	BaYMV ^b		
	rym3	5HS	Ea 52, Ishuku Shirazu	BaYMV, BaYMV-2	BaYMV ^b	5.8 5.8 6.4	
	rym4	3HL	Ragusa, Franka	BaMMV, BaYMV	BaMMV, BaYMV	HVM74 EBmac0639 Bmac0018 Bmag0009	
Hv-elF4E	rym5	3HL	Mokusekko 3, Resistant Ym No. 1, W122/37.1	BaMMV, BaYMV, BaYMV-2	BaMMV, BaYMV, BaYMV-2, BaYMV ^b	Brnac0127 Brnag0867 Brnag0496 HVM14	-19.0
	rym6	3HL	Prior, Amagi Nijo	susceptible	BaYMV ^b		18.0 Bmag0496
	rym7	1HS	HHor 3365	BaMMV	BaMMV	Bmag0001 / 9.3 Bmag0870 / 10.3	
	rym8	4HL	10247	BaMMV, BaYMV	BaMMV	EBmac0806 21.4	
	rym9	4HL	Bulgarian 347	BaMMV	BaMMV		22.0 EBmac0874
	rym10	3HL	Hiberna	BaYMV, BaYMV-2	BaYMV, BaYMV-2		23.0 EBmac0639
HvPDIL5-1	rym11	4HL	Russia 57	BaMMV, BaYMV, BaYMV-2	BaMMV		24.0 // Bmac0018 Bmag0009 HVM14 Bmac0127
	rym12	4HL	Muju covered 2	BaMMV, BaYMV, BaYMV-2	BaMMV		25.0 Bmac0001
	rym13	4HL	Taihoku A	BaMMV, BaYMV, BaYMV-2	BaMMV		25.0 · · · · Bmagooor
	Rym14 ^{Hb}	6HS	H. vulgare cv `Borwina' × H. bulbosum hybrid	BaMMV, BaYMV-1, -2	BaMMV, BaYMV		
	rym15	6HS	Chikurin Ibaraki 1	BaMMV	BaMMV		40.00 EBmac0806
	Rym16 ^{Hb}	2HL	H. vulgare cv `Borwina' × H. bulbosum hybrid	BaMMV, BaYMV-1, -2	BaMMV, BaYMV-1, -2		
	Rym17	3H	six-rowed barley PK23-2	BaYMV Japanese strains I, III	BaYMV Japanese strains I, III	Genetic map of barley	chromosome 6H
	Rym18	4H	six-rowed barley PK23-2	BaYMV Japanese strains I, III	BaYMV Japanese strains I, III	Including BaMMV resis	tance gene rym15
	eIF4E HOR3298	3HL	Iranian barley landrace 'HOR3298'	BaMMV, BaYMV	BaMMV, BaYMV		

Le Gouis et.al, 2004

Sequence changes of some European strains of BaYMV/BaMMV overcome the rym4 or rym5 mediated resistance. (Habekuß et al. 2008; Kühne et al. 2003; Kanyuka et al. 2004)

BaYMV isolates were detected in winter barley in Poland and Iran, where the disease was not yet reported. (Hosseini et al. 2014; Jezewska and Trzmiel 2009)

Introduction



Aims of project

- i. resolve the discrepancy between the two maps,
- ii. construct a high resolution mapping population of BaMMV resistance gene rym15,
- iii. narrow down the target region,
- iv. saturate the map with the final aim to isolate rym15.



First attempt towards fine mapping



In addition to a non-colinear order at previous maps, mechanical inoculation of around 200 F_2 plants from Chikurin Ibaraki 1 × Uschi cross revealed a large number of resistent progenies, parental lines used for screening were heterozygous, and the first map was distorted.

Map-based cloning strategy for BaMMV resistance gene rym15

Aims	Steps	Plant materials	Time schedule
	low-resolution mapping	217 DH lines (Chikurin Ibaraki 1 × Plaisant)	Le Gouis et al., 2004
Mapping of gene/s	medium-resolution mapping	522 F ₂ plants – F ₃ families Igri × Chikurin Ibaraki 1; Chikurin Ibaraki 1 × Uschi	2 years (2017-2018)
Isolation of chromosomal region containing the gene (F ₂ based)	high-resolution mapping	423 F_4 RILs derived from ~8000 F_2 plants Igri × Chikurin Ibaraki 1; Chikurin Ibaraki 1 × Uschi	3 years (2019-2021)

Igri (S) × Chikurin Ibaraki 1 (R) 2260 F_2 seeds

Chikurin Ibaraki 1 (R) × Uschi (S) 5813 F_2 seeds

Medium-resolution mapping

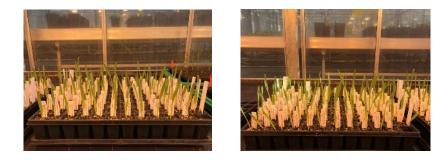


 F_2 population Igri (S) × Chikurin Ibaraki 1 (R)





F₂ population Chikurin Ibaraki 1 (R) × Uschi (S)



F₂ plants from cross Igri × Chikurin Ibaraki 1

Cross	Plant (F ₁)	Number of seeds (F ₂)		
Igri/ <mark>1 ×</mark> Chikur. Ibar. 1	1	154		
	2	159		
	3	56		

F₂ plants from cross Chikurin Ibaraki 1 × Uschi

Cross	Plant (F ₁)	Number of seeds (F ₂)
Chikur. Ibar. 1/1 × Uschi	1	115
	2	71
	3	120
	4	65

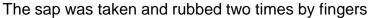
Medium-resolution mapping

Mechanical inoculation and ELISA



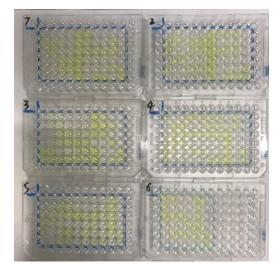






Pfl.	Bon.	30'	60'	BaMMV
3_1	+?	0.01	0.03	r
3_2	+?	0.47	0.99	S
3_3	+?	0.01	0.02	r
3_4	+	1.04	1.68	S
3_5	+	1.12	1.71	S
3_6	+	0.88	1.57	S
3_7	-	0.01	0.03	r
3_8	+	0.22	0.49	S
3_9	+	0.73	1.43	S
3_10	(+)	0.70	1.40	S
3_11	-	0.00	0.01	r

Plants with an extinction E405 > 0.10 were qualitatively scored as susceptible



S.G. Weeks after fisr

60 min after addition of p-Nitrophenyl Phosphate (PNPP) substrate buffer

Phenotypic analysis of F₂ plants and F₃ families

Segregation ratio and Chi-square test analysis of F_2 and F_3 progenies from two F_2 populations inoculated with BaMMV

Generation	Susceptible	Segregating	Resistant	Total	Expected ratio	X²	P value
F ₂ ª_family1	<u>103</u>	<u>-</u>	<u>44</u>	<u>147</u>	<u>3:1</u>	<u>1.907</u>	<u>0.1673</u>
F ₃ ª_family1	<u>39</u>	<u>64</u>	<u>44</u>	<u>147</u>	<u>1:2:1</u>	<u>2.796</u>	<u>0.2471</u>
F ₂ ª_family2	<u>109</u>	=	<u>36</u>	<u>145</u>	<u>3:1</u>	<u>0.002</u>	<u>0.9643</u>
<u>F₃ª_family2</u>	<u>25</u>	<u>84</u>	<u>36</u>	<u>145</u>	<u>1:2:1</u>	<u>5.317</u>	<u>0.0701</u>
<u>F₂ª_family3</u>	<u>38</u>	=	<u>12</u>	<u>50</u>	<u>3:1</u>	<u>0.027</u>	<u>0.8695</u>
<u>F₃ª_family3</u>	<u>10</u>	<u>28</u>	<u>12</u>	<u>50</u>	<u>1:2:1</u>	<u>0.880</u>	<u>0.644</u>
<u>F₂ª_total</u>	<u>250</u>	=	<u>92</u>	<u>342</u>	<u>3:1</u>	<u>0.659</u>	<u>0.4169</u>
F ₃ ª_total	<u>74</u>	<u>176</u>	<u>92</u>	<u>342</u>	<u>1:2:1</u>	<u>2.187</u>	<u>0.335</u>
F ₂ ^b _family1	97	-	19	116	3:1	4.598	0.0320*
F ₃ ^b _family1	41	56	19	116	1:2:1	8.483	0.0144 *
F ₂ ^b _family2	43	-	21	64	3:1	2.083	0.1489
F ₃ ^b _family2	12	31	21	64	1:2:1	2.594	0.2734
F ₂ ^b _total	140	-	40	180	3:1	0.741	0.3893
F ₃ ^b _total	53	87	40	180	1:2:1	2.078	0.3538

^a_progenies derived from cross of Igri 1 × Chikurin Ibaraki 1

^b progenies derived from cross of Chikurin Ibaraki 1 × Uschi

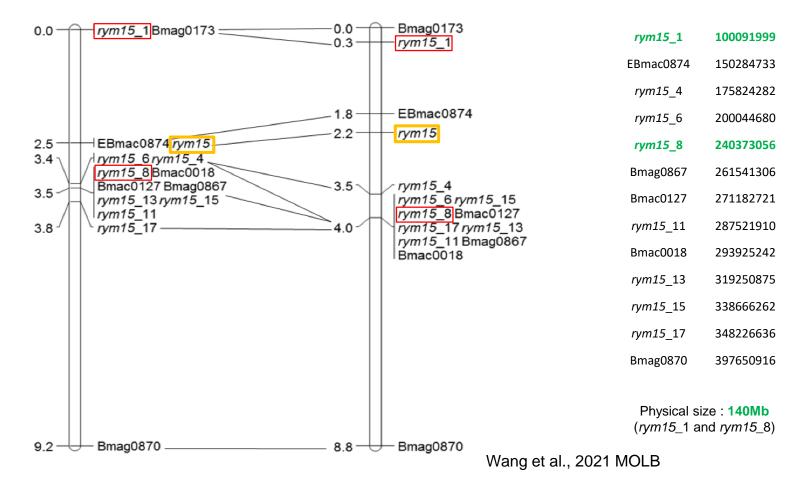
* the ratio not fit to expected ratio

BaMMV resistance in Chikurin Ibaraki 1 is controlled by a single recessive resistance gene *rym15*

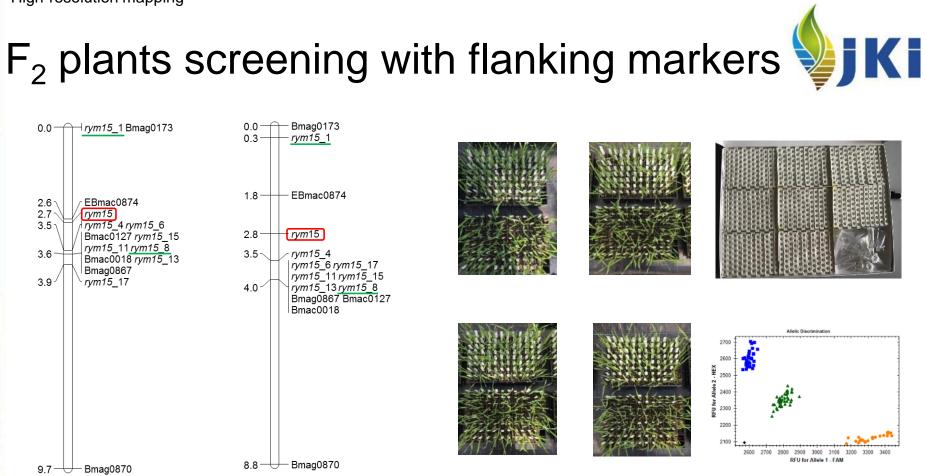
Medium-resolution mapping

Co-linearity of medium-resolution maps





Medium-resolution maps of BaMMV resistance gene *rym15 that* were constructed based on analysis of 342 and 180 F_2 plants derived from the crosses Igri × Chikurin Ibaraki 1 (left) and Chikurin Ibaraki 1 × Uschi (right) allowed selection of flanking markers at distance of about 3 cM.



Procedure of screening (each time 8 plates)

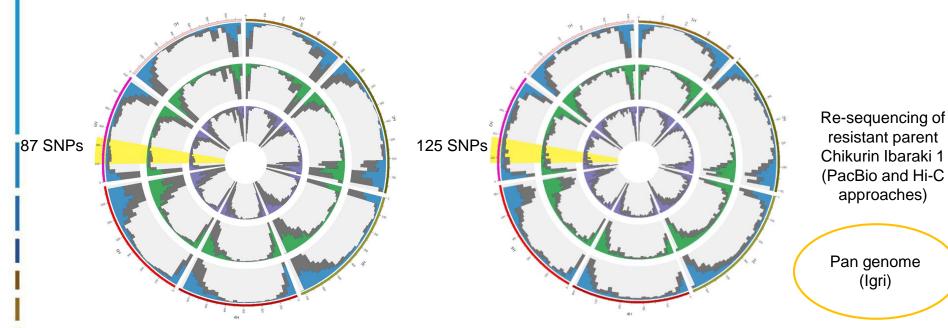
Screening of F₂ plants for the construction of a high resolution mapping

Crosses	Number of analyzed E. planta	Number of recombinant F ₂ plants	Pasambination fraguancy	Number of segmental RILs (F ₄)		χ^2 1:1 (df=1, p > 0.05)
Crosses	Number of analyzed F ₂ plants	Number of recombinant F ₂ plants	Recombination nequency	Resistant	Susceptible	χ 1.1 (ul=1, p > 0.03)
Igri × Chikurin Ibaraki 1	2174	162	3.72%	67	72	0.18 (P=0.6714)
Chikurin Ibaraki 1 × Uschi	5728	288	2.51%	140	144	0.056 (P=0.8129)

SNPs distribution among GBS and 50K

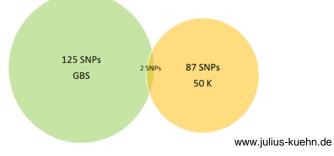


(Igri)



Landscape of the 50K (left) and GBS (right) polymorphisms

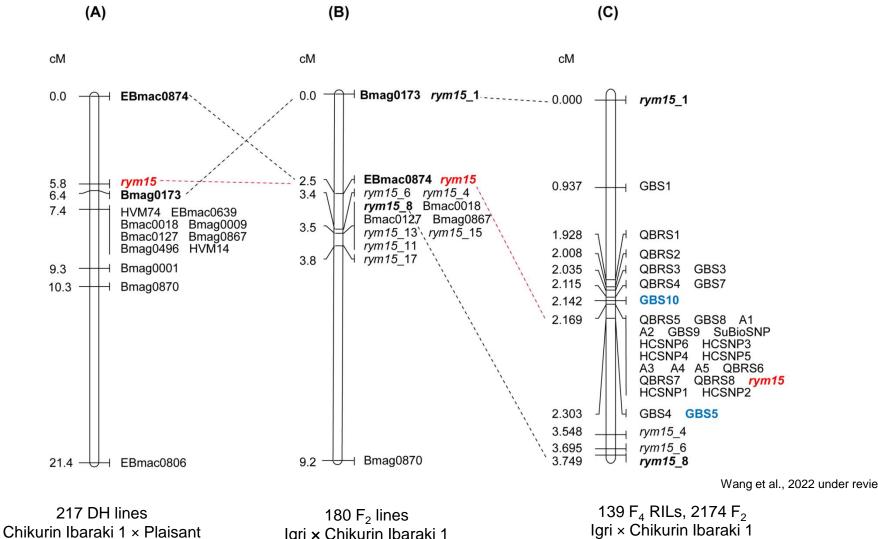
Grey, all SNPs from 50K chip. Blue, SNPs between Chikurin Ibaraki 1 and Igri. Green, SNPs between Chikurin Ibaraki 1 and Uschi. Purple, Same SNPs from two comparison (Chikurin Ibaraki 1 and Igri; Chikurin Ibaraki 1 and Uschi). Yellow, The interval between flanking markers rym15_1 and rym15_8.



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Igri x Chikurin Ibaraki 1 maps





Le Gouis et al. 2004

Igri x Chikurin Ibaraki 1 Medium-resolution, 2019 Wang et al., 2022 under review

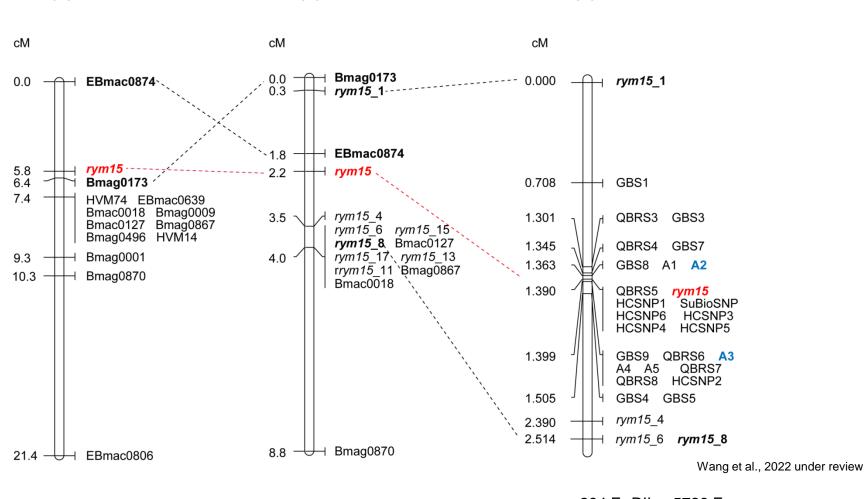
High-resolution, 2021

(A)

Chikurin Ibaraki 1 x Uschi maps

(B)



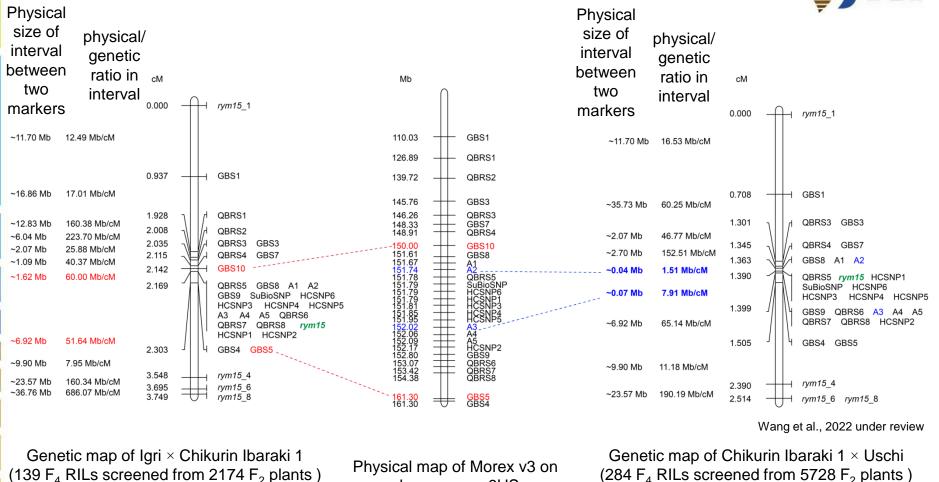


217 DH lines Chikurin Ibaraki 1 × Plaisant Le Gouis et al. 2004 $342 F_2$ lines Chikurin Ibaraki 1 × Uschi Medium-resolution, 2019 $\begin{array}{c} \text{284 F}_{4} \text{ RILs, 5728 F}_{2} \\ \text{Chikurin Ibaraki 1 \times Uschi} \\ \text{High-resolution, 2021} \end{array}$

(C)

Physical maps





Recombination frequency: 7.95-666.07 Mb/cM Interval (0.161 cM; 11.3 Mb)

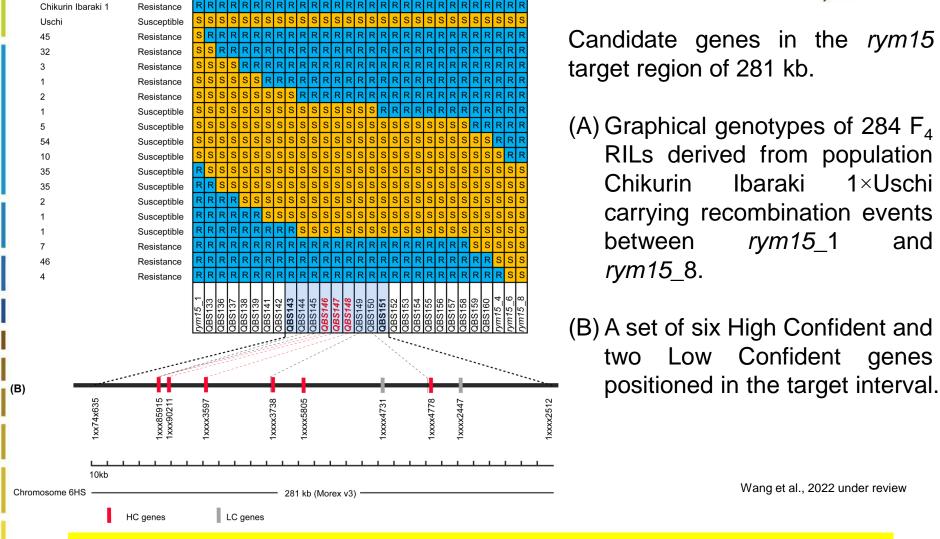
chromosome 6HS

Recombination frequency: 1.51-190.19 Mb/cM Interval (0.036 cM; 0.28 Mb)

The locus was downsized from 137 Mb to 0.28 Mb

Graphical genotypes of 284 F₄ RILs

(A) Parents/Number of RILs Reaction type Genotypes



Re-sequencing of candidate genes revealed polymorphisms at 3 HC genes



High-resolution mapping



HC genes	other 4 cultivar -> Chikurin	Chikurin Ibaraki 1	lgri	Uschi	Morex	Golden promise
HC1 (1080 bp)	TCA -> GCAS (Ser) -> A (Ala)	565 (G)	565 (T)	565 (T)	565 (T)	565 (T)
HC2 (636 bp)	AAC -> GACN (Asn) -> D (Asp)	316 (G)	316 (A)	316 (A)	316 (A)	-
HC3 (1026 bp)	TG G -> TG A W (Trp) -> * (Ter)	1026 (A)	1026 (<mark>G</mark>)	1026 (<mark>G</mark>)	1026 (<mark>G</mark>)	1026 (G)

HC1 Chikurin Ibaraki1 (3509 bp) Igri, Uschi, Golden Promise (3452 bp)



HC2 Chikurin Ibaraki1 (1092 bp) Igri, Uschi, Golden Promise (1091 bp)



HC3 Chikurin Ibaraki1 (1346 bp) Igri, Uschi, Golden Promise (1349 bp)



HC2 is identical between Chikurin Ibaraki 1 and Golden Promise

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Summary



- 1. Medium-resolution procedure resolved: distorted maps, marker non-colienearity and gene inheritance
- 2. Constructed medium-resolution maps, identified flanking markers rym15_1 and rym15_8 at ~ 3cM distance
- 3. Constructed 423 F_4 RILs Igri × Chikurin Ibaraki 1 (139 RILs screened from 2174 F_2 plants) Chikurin Ibaraki 1 × Uschi (284 RILs screened from 5728 F_2 plants)
- 4. Constructed high-resolution maps, downsized interval to 0.28 Mb in population Chikurin Ibaraki 1 × Uschi
- 5. Two HC candidate genes were identified

Next steps

- 1. Functional analysis/gene editing of two HC candidate genes: IPK
- 2. Allele mining and phylogenetic analysis of *rym15*
- 3. Gene expression analysis and screening TILLING population for two HC genes

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