Quantitative Trait Locus analysis and fine mapping for *Fusarium oxysporum* disease resistance in *Raphanus sativus* L. using GRAS–Di technology

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Introduction

- Radish faces a major threat from *Fusarium oxysporum* f. sp. *raphani* . The most effective approach for managing this disease is through breeding yellow resistant (**YR**) radish varieties.
- **Kaneko et al. (2007)** identified a YR-QTL on chromosome R7.
- **Yu et al. (2013)** expanded this research, discovering 8 QTLs (*qFW1-qFW8*) distributed across 5 linkage groups (LGs). QTL *qFW4* on LG3 (chromosome R5) was recognized as a major locus, *Fwr1*. Yu et al. (2020) fine-mapped *Fwr1* and found a candidate gene of RLK.
- **Ma et al. (2021)** detected YR-QTLs on chromosomes R7 and R9 and suggested potential resistance-related genes.
- **Lee et al. (2021)** identified QTLs, including one QTL co-located with an R7 SNP from a GWAS study.
- Three research groups identified a significant **YR-QTL in radishes on the R7 chromosome**, but it remains undetermined whether these QTLs are identical across different research groups due to limited molecular data on marker sequences.
- No one did fine mapping of the YR-QTL on chromosome 7 (R7-QTL).

Objectives

- Identification of the Quantitative Trait Locus (QTL) responsible for YR in radish.
- Fine mapping of the R7-QTL to identify the gene responsible for yellow resistance in radish.
- Validation of the usefulness of the GRAS–Di map in radish.

Materials and methods

F1

Inbred line

RK15–1 (Res) x AKM (Sus)

Production of F2:3 lines (n=132)

Inoculation test F2:3 lines

GRAS-Di （Genotyping by Random Amplicon Sequencing-Direct) Linkage map construction (Antmap ver. 1.2)

QTL analysis（Windows QTL Cartographer）

Map based cloning

Select F2:3 lines with chromosomal recombination in the target region (R7)

・Collecting Illumina short read sequences from both parental lines

・Creating CAPS and Indel markers.

Inoculation testing and genotyping of F2:3 lines with chromosomal recombination

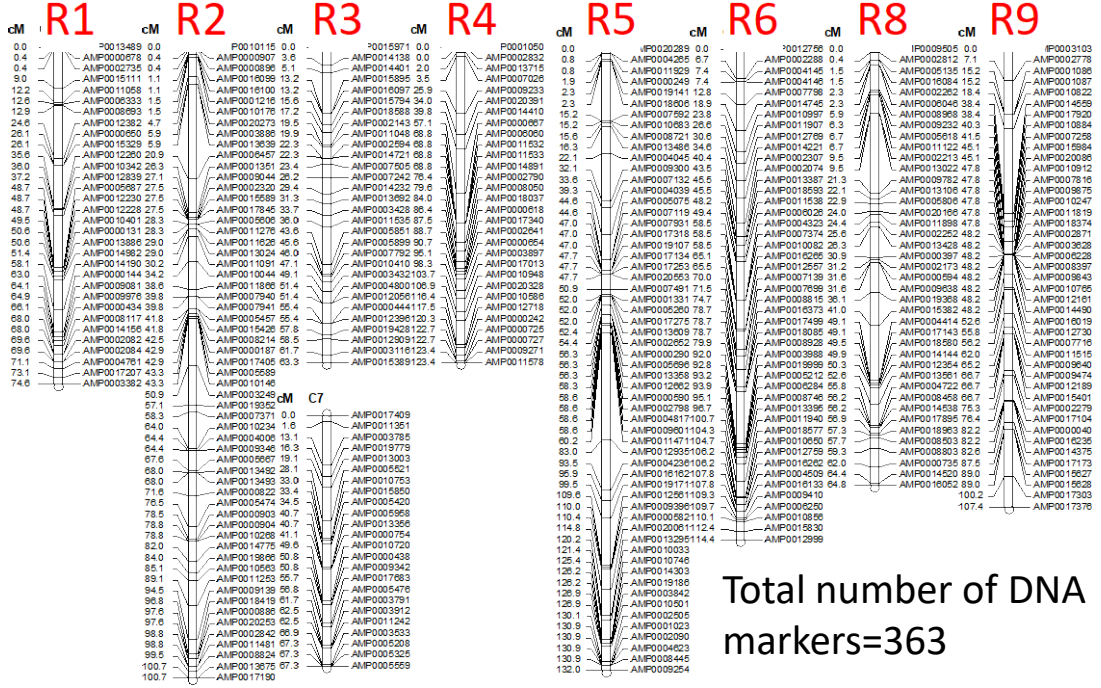
Collecting Nanopore sequences for the RK15 line's target region (R7) and RNA-seq analysis of both parental lines.

Identification of candidate genes and gene characterization

Results GRAS-Di Linkage map construction

（Antmap ver. 1.2）

co-dominant marker GRAS-Di map.



Total number of DNA markers=363

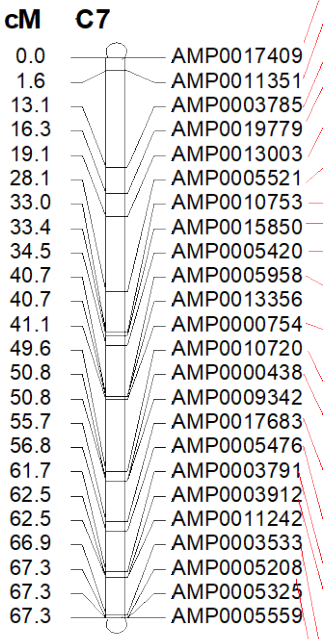
Total number of DNA markers used in the three maps.

Linkage map	No. of DNA markers
Co-dominant map	363
co-dominant＋female-derived map	702
co-dominant＋male-derived map	736

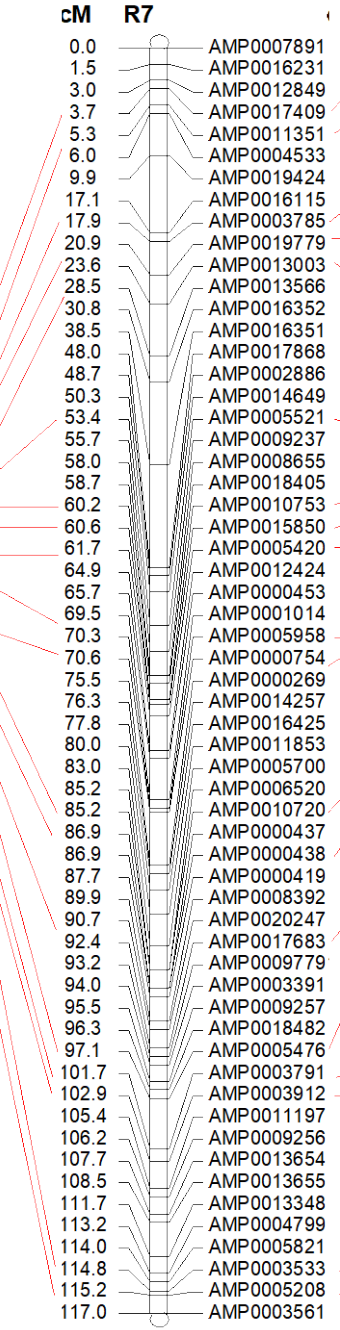
Knapp et al. (1995) found that repulsion-dominant markers are imprecisely mapped. They proposed a method to create two pure-coupling F2 maps by segregating dominant markers based on their female or male origin.

— indicate anchor co-dominant markers.

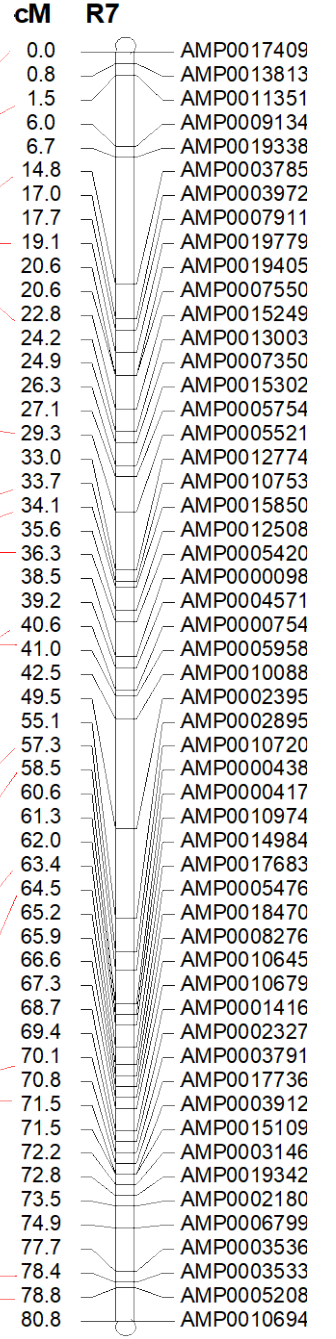
co-dominant marker map



co-dominant＋ male-derived map



co-dominant＋ female-derived map

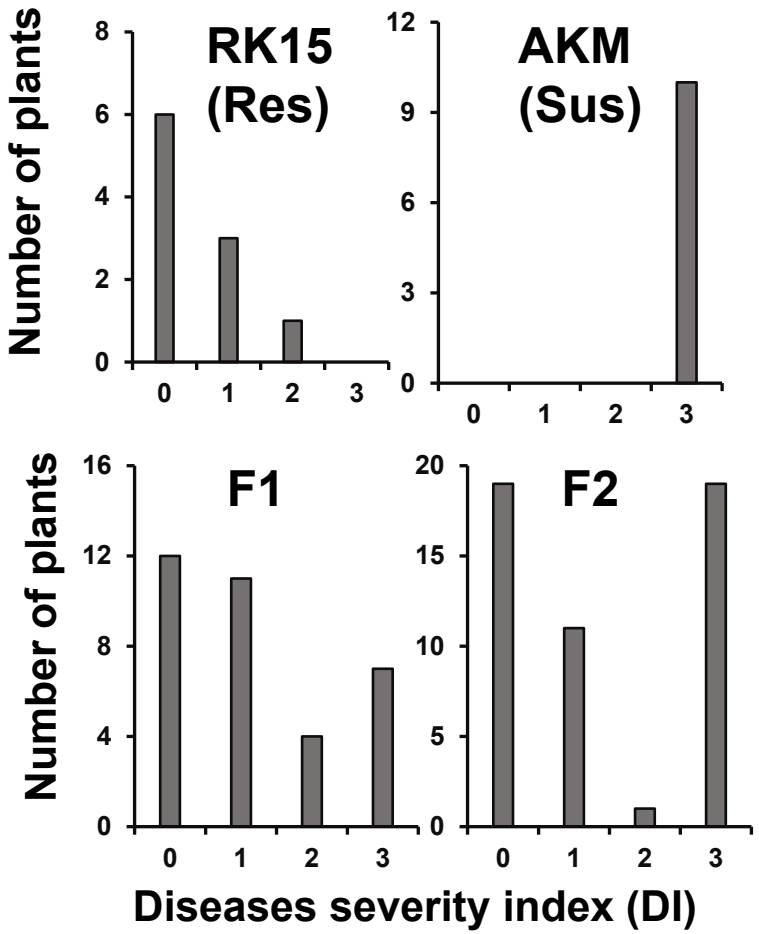


Results

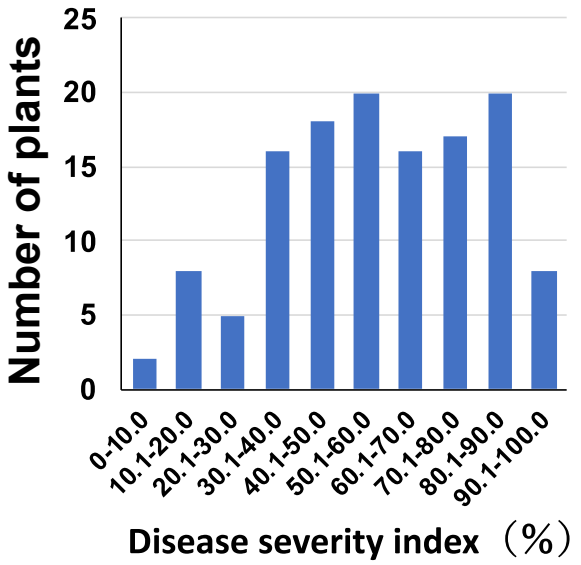
F2:3 line (10 seedlings per line)

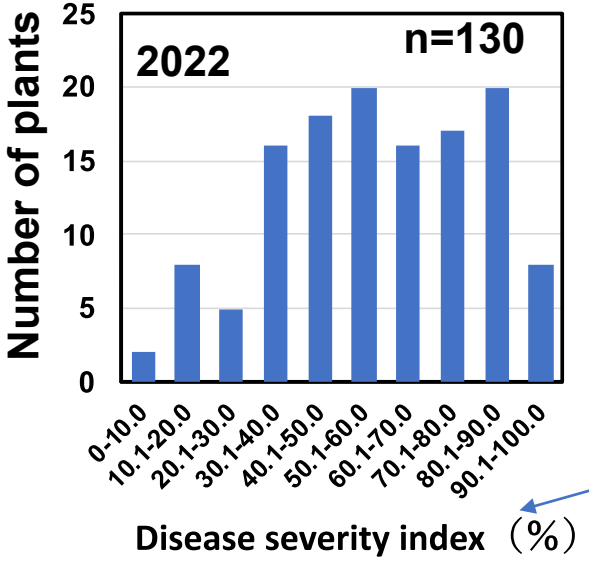
Fusarium oxysporum* f. sp. *raphani （MAFF731043）

Direct seed sowing method (Growth room)



Pipetting method (Greenhouse)





The conidia filtrate (**2x10^{5}cfu**) was added per 1g of soil. Disease index were observed one month later after inoculation.

DI=0 No symptom

DI=1 Slight yellowing

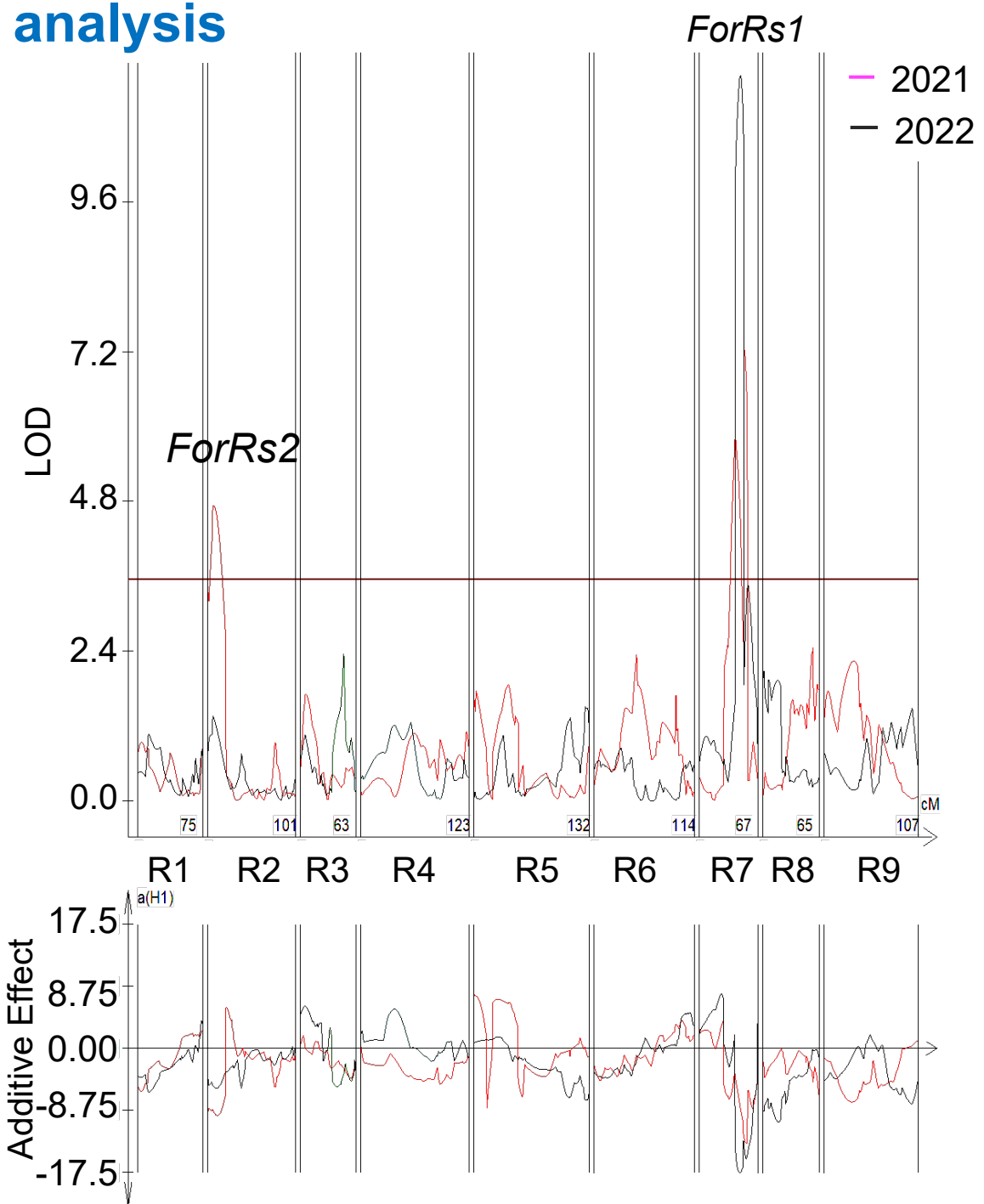
DI=2 Deep yellowing and loss of leaves/ atrophy

DI=3 Death of plant

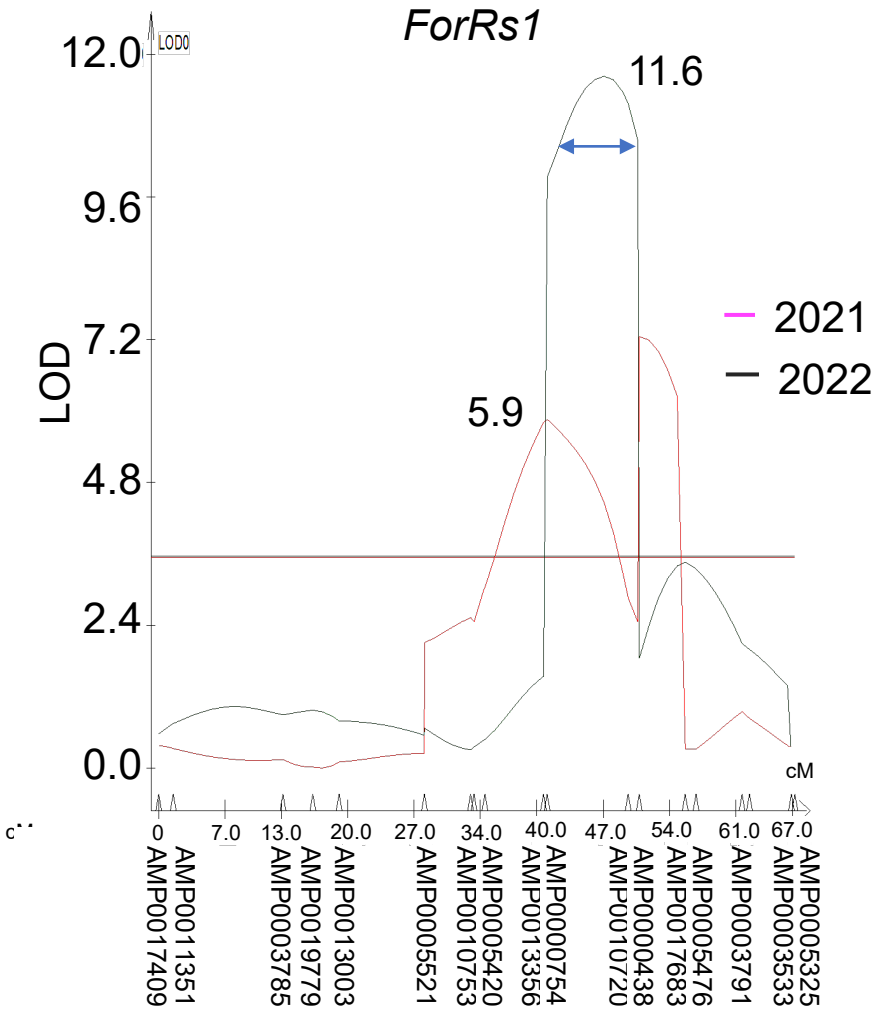
Percentage of DI (PoDI) was estimated as follows,

DI =Σ (DI × X) ×100/ N × 3, where X= number of plants with symptom in each line, N= total number of seedlings.

QTL analysis



R^2 x100= 2021 (12.6%)
2022 (33.0%)



QTL confirmation using selected selfed F2 lines

F_2-13: Aabb F_2-80: AaBB F_2-50: aaBb F_2-95: AABb

***ForRs1* (Major QTL)**

Resistant parent allele = A
Susceptible parent allele = a

***ForRs2* (Minor QTL)**

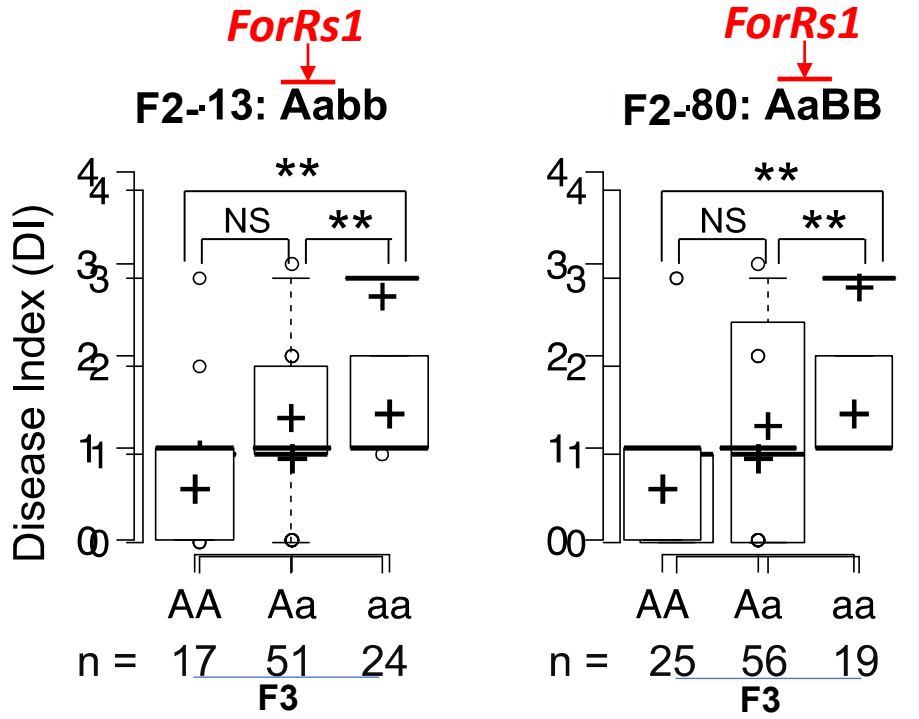
Res parent allele = B
Sus parent allele = b

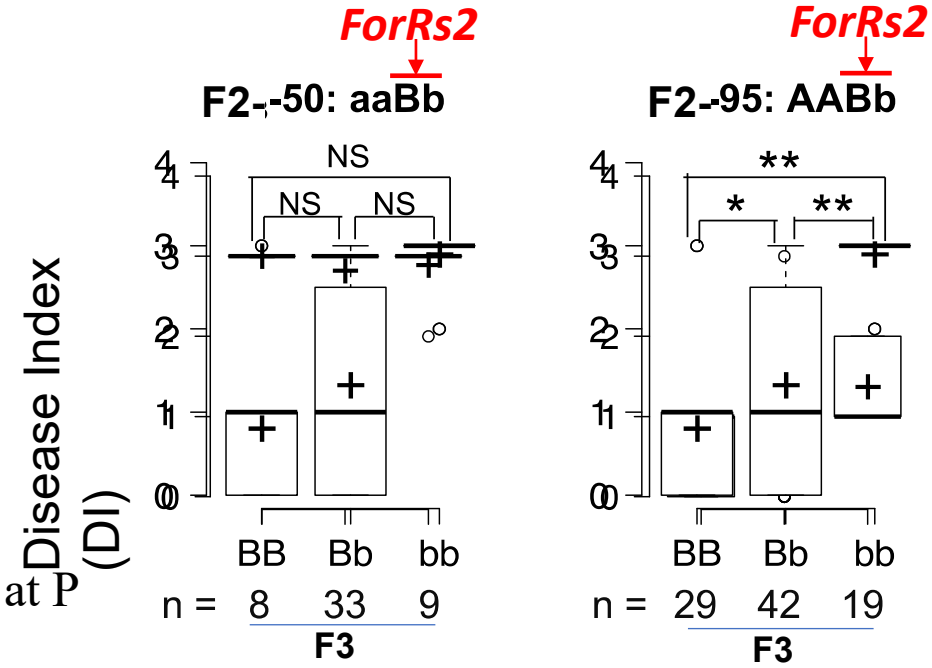
Genotyped using **AMP0000754** and **AMP0003886** markers, which are the flanking markers of *ForRs1* and *ForRs2*, respectively.

⊗

F3 line

Direct seed sowing method (Growth room, 28 ℃)





*, ** and NS indicate statistically significant at P ≤ 0.05, 0.01 and not significant, respectively.

Selection of recombinant individuals in the ForRs1 region (1)

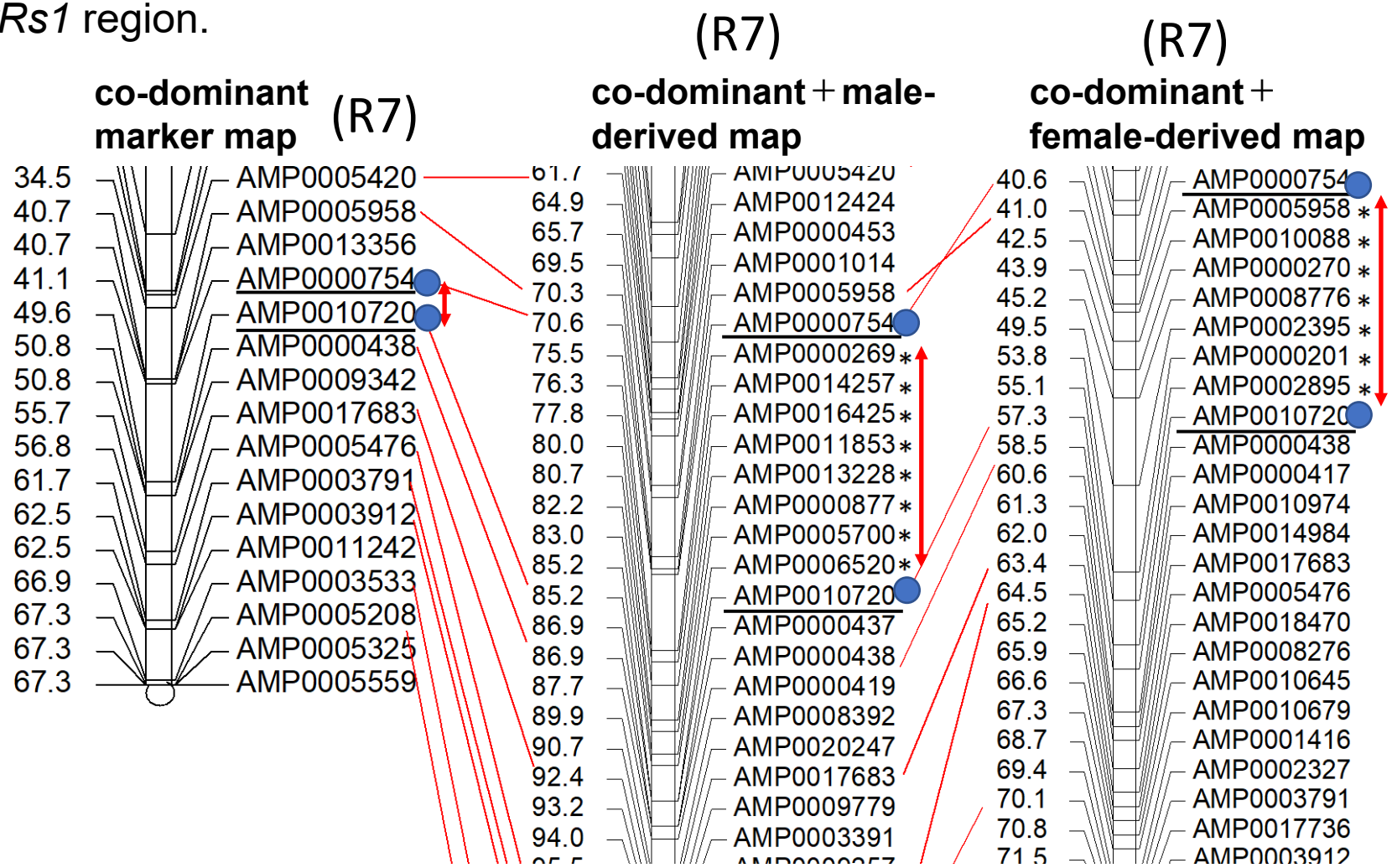
Among the 132 F2 plants, the *ForRs1* flanking GRAS-Di markers, AMP0000754 and AMP0010720, identified 18 F2 plants with recombinants in the *ForRs1* region.

Further selection

F2 recombinant plants-
28, 46, 69, 15, 52, 68

The high-density GRAS-Di maps effectively positioned multiple dominant markers between two co-dominant markers in the *ForRs1* region on the R7 chromosome, which was **very useful for selecting recombinants with distinct recombination points in the target region.**

Enlarged ForRs1 region of the R7 GRAS-Di map



Underlined markers are co- dominant markers that flank ***ForRs1*** region.

— indicate anchor co-dominant markers.

Selection of recombinant individuals in the ForRs1 region (2)

F2 recombinant plants-28, 46, 69, 15, 52, 68

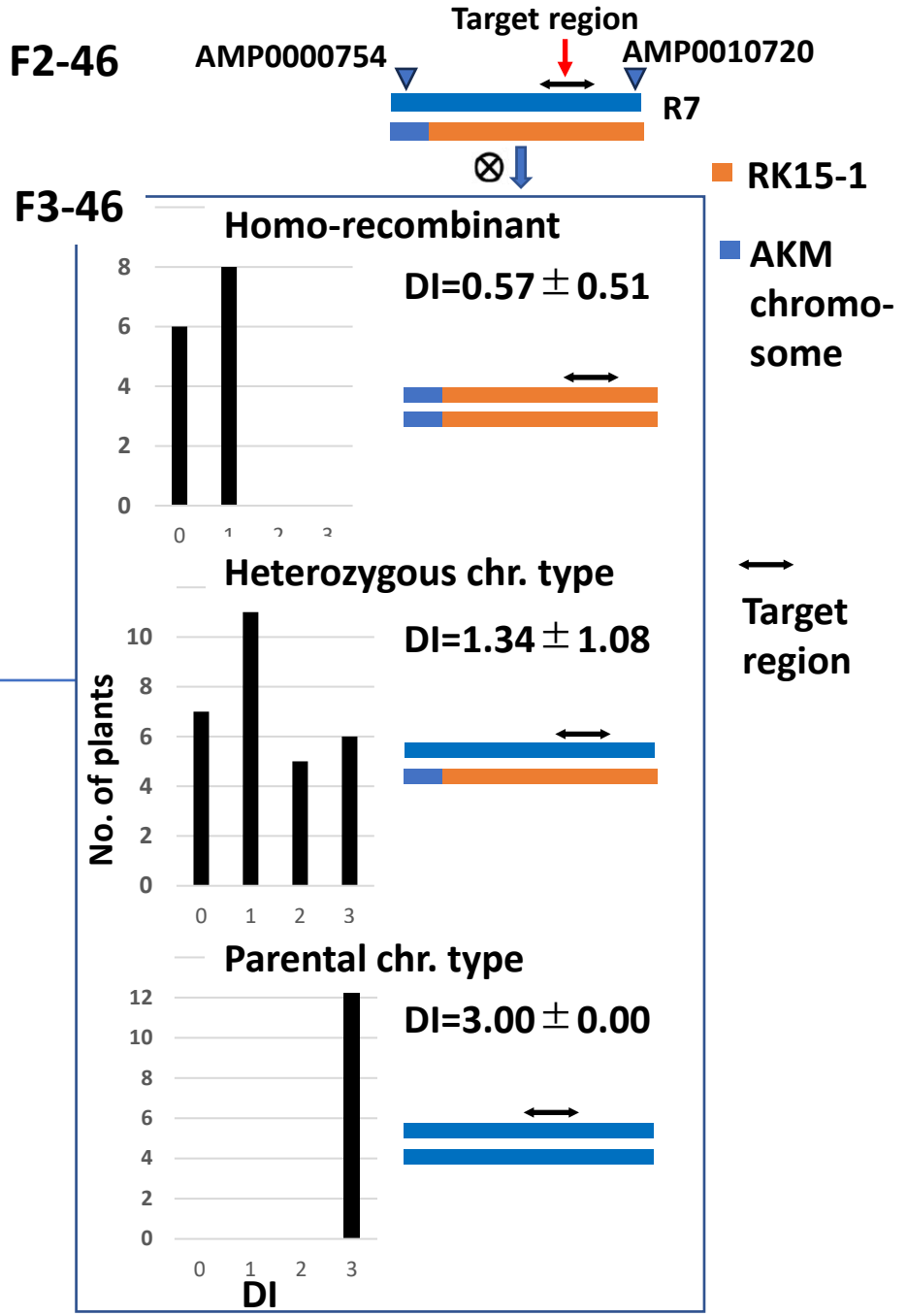
⊗

F2:3 line-28, 46, 69, 15, 52, 68

Genotyping at the ForRs1 specific markers and phenotyping

The numbers represent the number of individuals, and the numbers in parentheses indicate disease severity.

F2:3 line	parental homo	hetero	recombinant homo
28 line	8 (1.00), RK15	23 (1.13)	13 (0.69)
46 line	13 (3.00), AKM	29 (1.34)	14 (0.57)
69 line	6 (0.83), RK15	19 (0.84)	8 (0.50)
15 line	11 (1.00), RK15	20 (0.95)	16 (0.94)
52 line	3 (3.00), AKM	8 (3.00)	4 (2.25)
68 line	15 (1.40), RK15	23 (2.35)	16 (3.00)
	recombinant homo A	hetero	recombinat home B
61 line	5 (2.86)	15(2.87)	15 (2.93)



Results of map-based cloning

- the reference genome
- Nanopore sequence
- Graphical genotypes of recombinant plants
  - RK15-1 (Res)
  - AKM (Sus)
- Genes involved in the delimited region

A

Candidate genes proposed at the YR-QTL (*FoRsR7.1*) identified by Ma et al. in 2021.

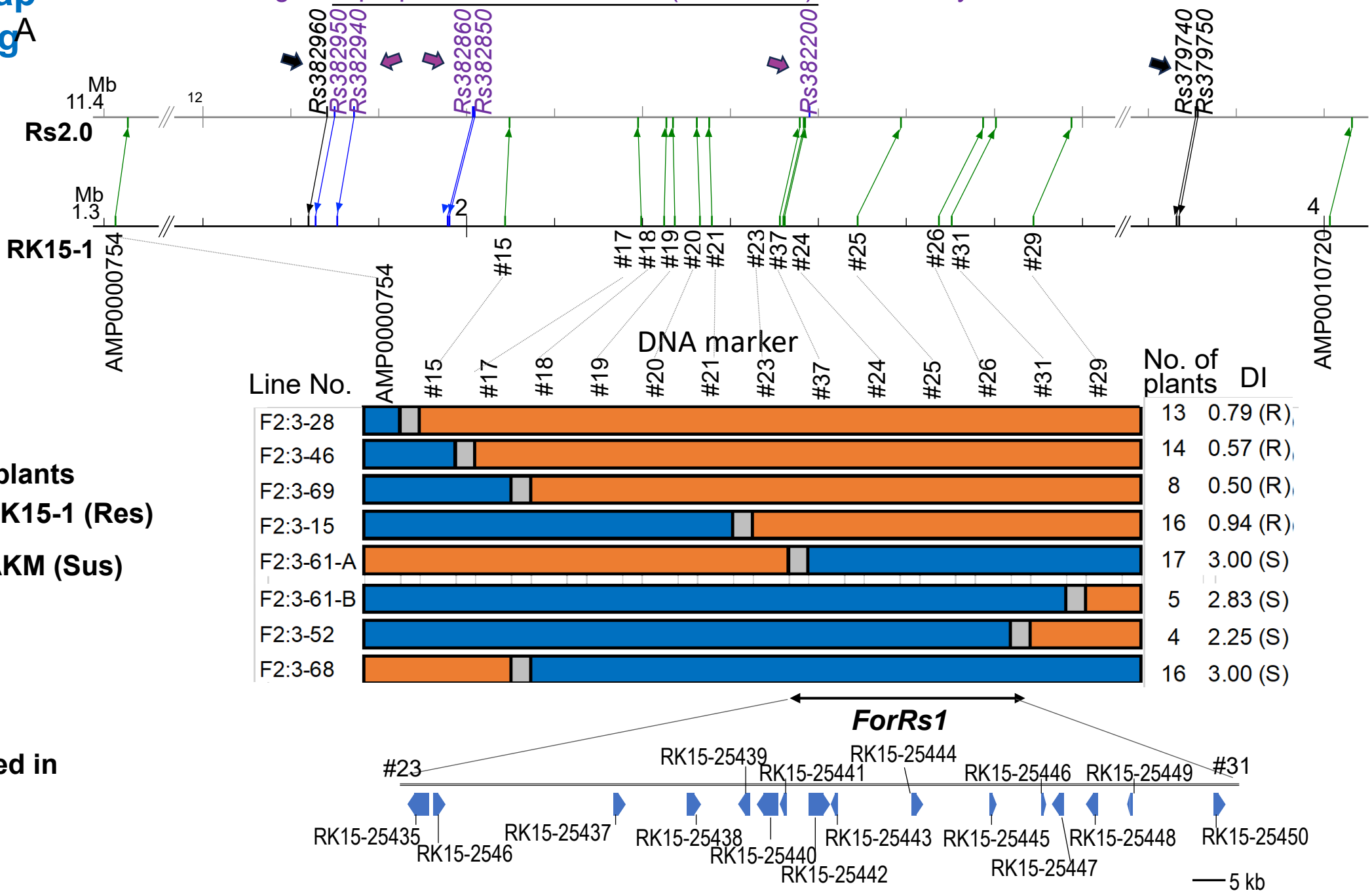


Table: Annotated genes in the fine-mapped region of *ForRs1* (R7)

	Serial No. of genes	Length (bp)	DNA strand	*A. thaliana* homolog	Gene annotation
1	**RK15.25435**	3,621	-	AT1G74190.1	**Receptor like protein 15**
2	**RK15.25436**	2,382	+	AT1G74210.1	Encodes a member of the glycerophosphodiester phosphodiesterase (GDPD) family.
3	**RK15.25437**	2,096	+	AT1G74210.1	Encodes a member of the glycerophosphodiester phosphodiesterase (GDPD) family.
4	RK15.25438	2,552	+	AT2G02220.1	**Encodes a protein interacting with phytosulfokine**
5	RK15.25439	1,105	-	AT1G07070	Ribosomal protein L35Ae family protein
6	**RK15.25440**	3,521	-	AT1G74310.1	Encodes ClpB1, which belongs to the casein lytic proteinase/heat shock protein 100 (Clp/Hsp100) family.
7	**RK15.25441**	1,195	-	AT1G74370.1	RING/U-box superfamily protein.
8	**RK15.25442**	3,382	+	AT1G74400.1	**Tetratricopeptide repeat (TPR)-like superfamily protein.**
9	RK15.25443	999	-	AT1G74420.2	Predicted fucosyltransferase, based on similarity to FUT1, but not functionally redundant with FUT1.
10	**RK15.25444**	2,026	+	AT1G74430.1	**Encodes a putative transcription factor (MYB95).**
11	RK15.25445	1,687	+	AT1G74430.1	Encodes a putative transcription factor (MYB95).
12	RK15.25446	674	+	AT1G74458	transmembrane protein
13	RK15.25447	1,871	-	AT1G74460.1	GDSL-motif esterase/acyltransferase/lipase.
14	**RK15.25448**	2,291	-	AT1G74490.1	**Protein kinase superfamily protein. PBL29, PBS1-LIKE 29**
15	RK15.25449	630	-	AT1G74500	basic helix-loop-helix protein 135, bHLH135
16	RK15.25450	2,197	+	AT1G74510.3	Galactose oxidase/kelch repeat superfamily protein.

Conclusion

- **The high-density GRAS-Di maps effectively positioned multiple dominant markers in each linkage group, and very useful for selecting recombinants with distinct recombination points in the target region.**

- **We detected YR-QTLs for radish wilt disease on chromosomes R2 and R7, which we named *ForRs2* and *ForRs1*, respectively.**

- **Through map-based cloning using F2:3 progeny, we narrowed down the candidate gene region to within 195 kb.**

- **Nanopore sequencing and RNA-seq analysis in the candidate region identified 16 expressed genes, including four candidate genes like LRR-RLP/RLK type genes.**

THANK YOU