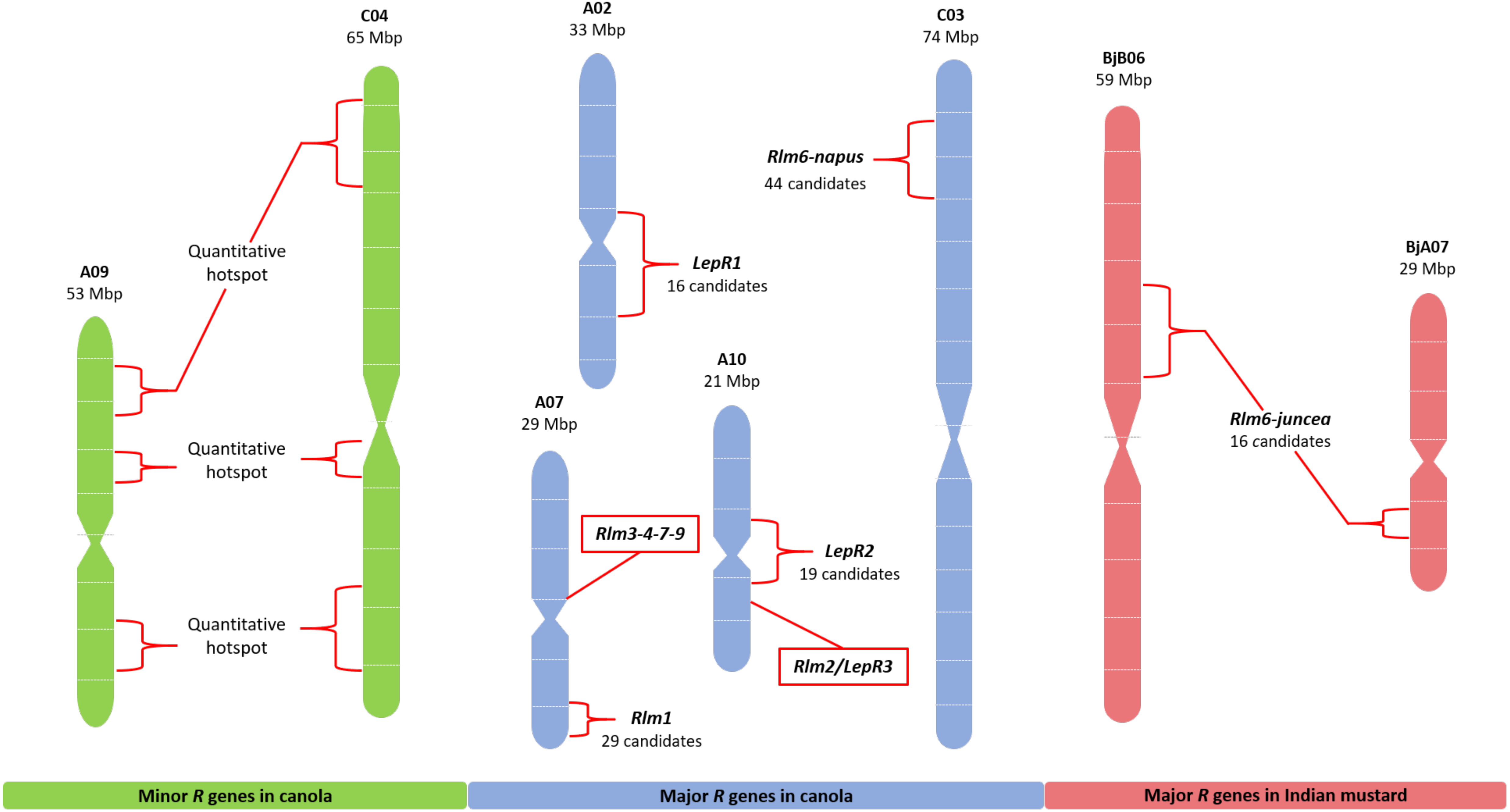
Program 3: Identification and characterisation of novel sources of blackleg resistance

Jacqueline Batley
School of Biological Sciences
University of Western Australia
jacqueline.batley@uwa.edu.au

Aims

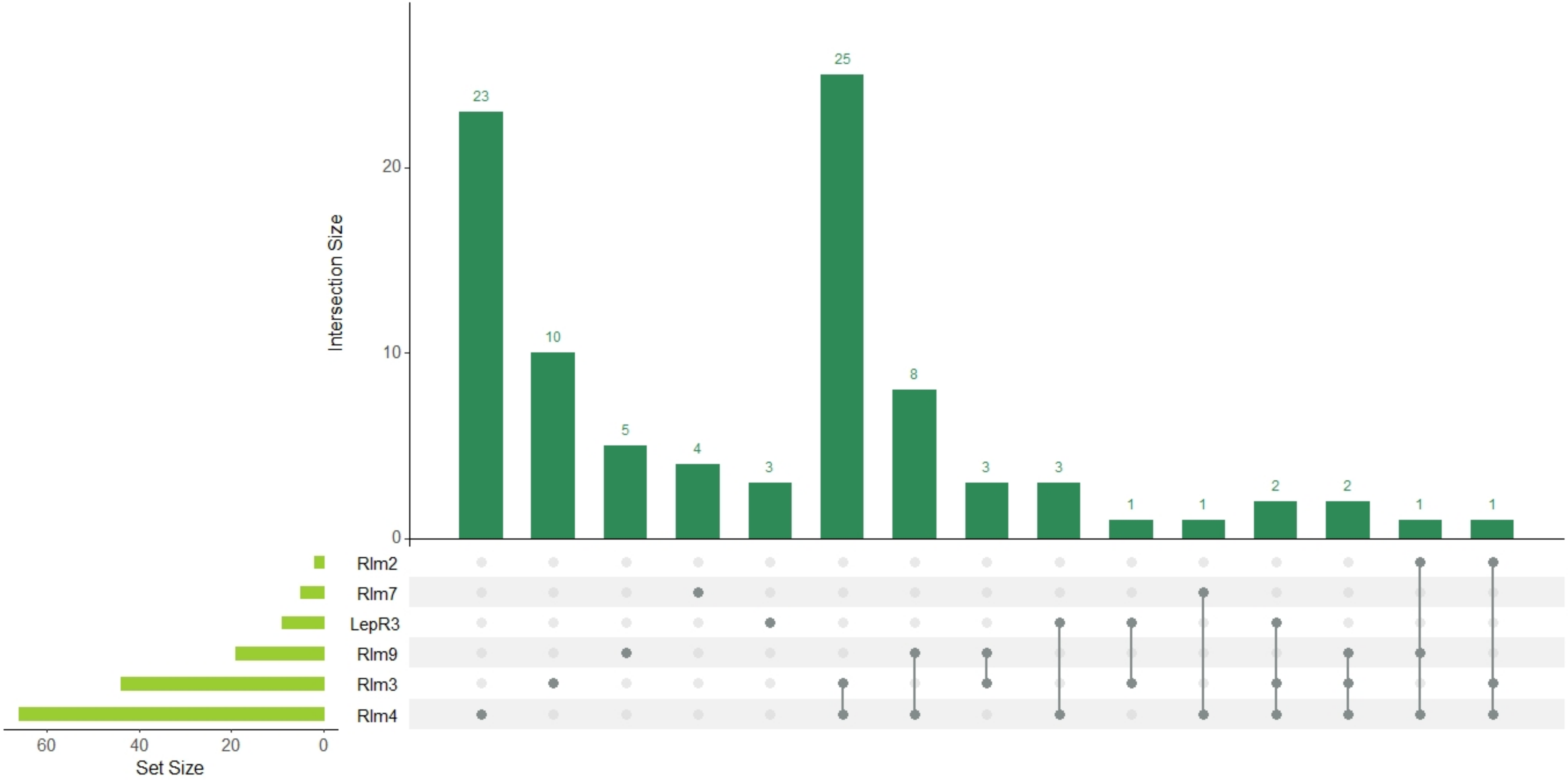
- Develop molecular markers for routine screening of blackleg resistance genes
- Identify candidate genes for genetically mapped resistance genes
- Deliver novel sources of qualitative blackleg resistance

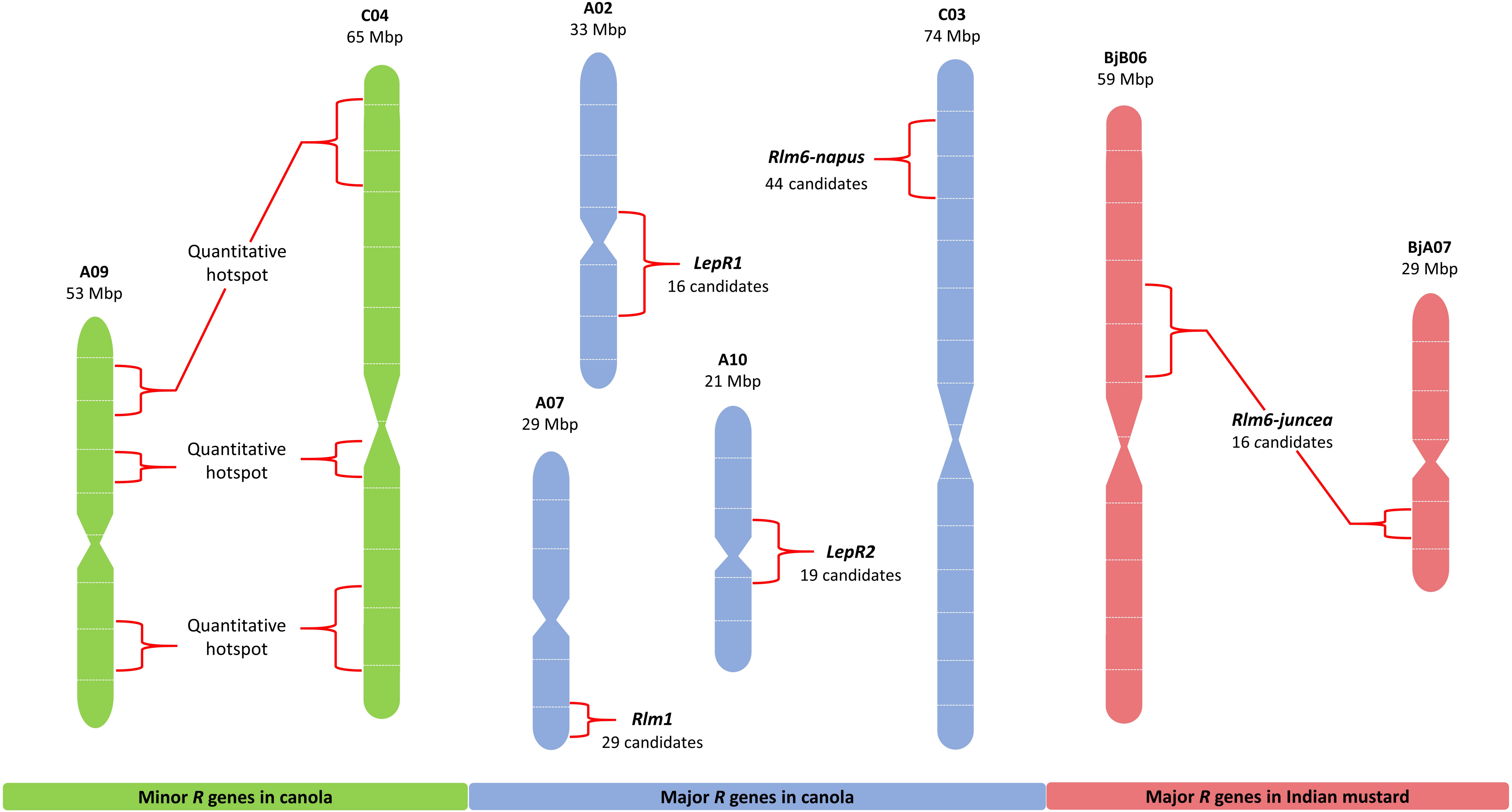

A09
53 Mbp
C04
65 Mbp
Quantitative hotspot
Quantitative hotspot
Quantitative hotspot
A02
33 Mbp
LepR1
16 candidates
A07
29 Mbp
Rlm3-4-7-9
Rlm1
29 candidates
A10
21 Mbp
LepR2
19 candidates
Rlm2/LepR3
C03
74 Mbp
Rlm6-napus
44 candidates
BjB06
59 Mbp
BjA07
29 Mbp
Rlm6-juncea
16 candidates
Minor *R* genes in canola
Major *R* genes in canola
Major *R* genes in Indian mustard

LepR3, Rlm 2, 3,4,7,9 screening

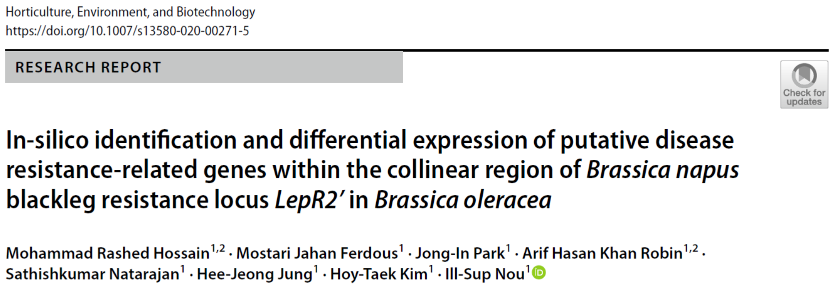
- Candidate genes identified for *Rlm 3, 4* and *7*
- *Rlm2, Rlm9* and *LepR3* cloned
- KASP markers for *LepR3, Rlm2, Rlm4* all working well
- KASP for *Rlm9* developed
- KASP *Rlm3* and *Rlm7* being optimised

Marker screening (*Rlm3-4-7-9*, *Rlm2-LepR3*)




A09
53 Mbp
C04
65 Mbp
Quantitative hotspot
Quantitative hotspot
Quantitative hotspot
A02
33 Mbp
LepR1
16 candidates
A07
29 Mbp
Rlm1
29 candidates
A10
21 Mbp
LepR2
19 candidates
C03
74 Mbp
Rlm6-napus
44 candidates
BjB06
59 Mbp
BjA07
29 Mbp
Rlm6-juncea
16 candidates
Minor *R* genes in canola
Major *R* genes in canola
Major *R* genes in Indian mustard

LepR2 Workflow

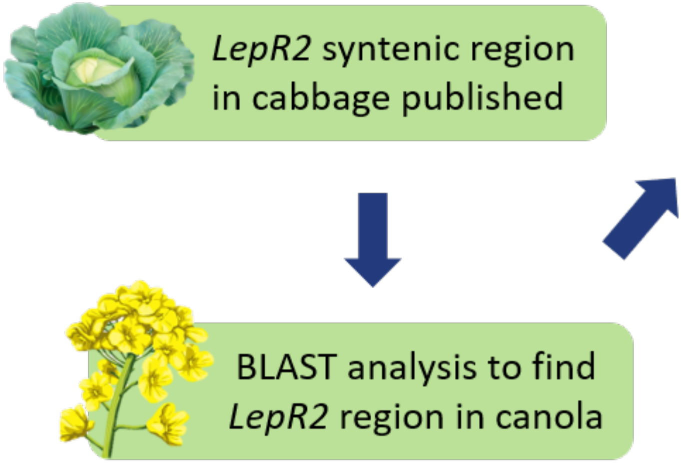


Horticulture, Environment, and Biotechnology
https://doi.org/10.1007/s13580-020-00271-5

RESEARCH REPORT

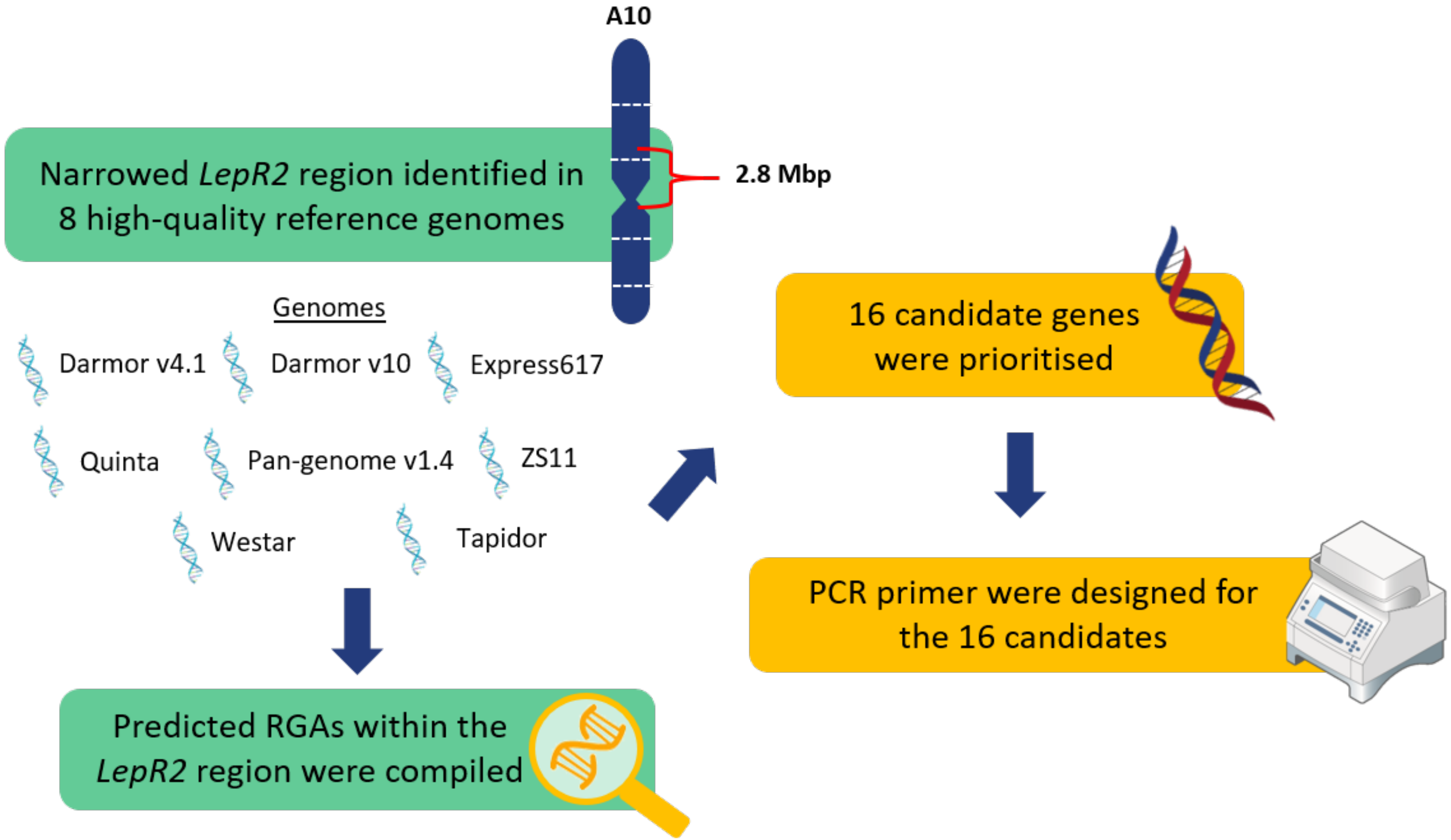
In-silico identification and differential expression of putative disease resistance-related genes within the collinear region of *Brassica napus* blackleg resistance locus *LepR2'* in *Brassica oleracea*

Mohammad Rashed Hossain[1,2] · Mostari Jahan Ferdous[1] · Jong-In Park[1] · Arif Hasan Khan Robin[1,2] · Sathishkumar Natarajan[1] · Hee-Jeong Jung[1] · Hoy-Taek Kim[1] · Ill-Sup Nou[1]



LepR2 syntenic region in cabbage published

BLAST analysis to find *LepR2* region in canola



Narrowed *LepR2* region identified in 8 high-quality reference genomes

Genomes

- Darmor v4.1
- Darmor v10
- Express617
- Quinta
- Pan-genome v1.4
- ZS11
- Westar
- Tapidor

Predicted RGAs within the *LepR2* region were compiled

16 candidate genes were prioritised

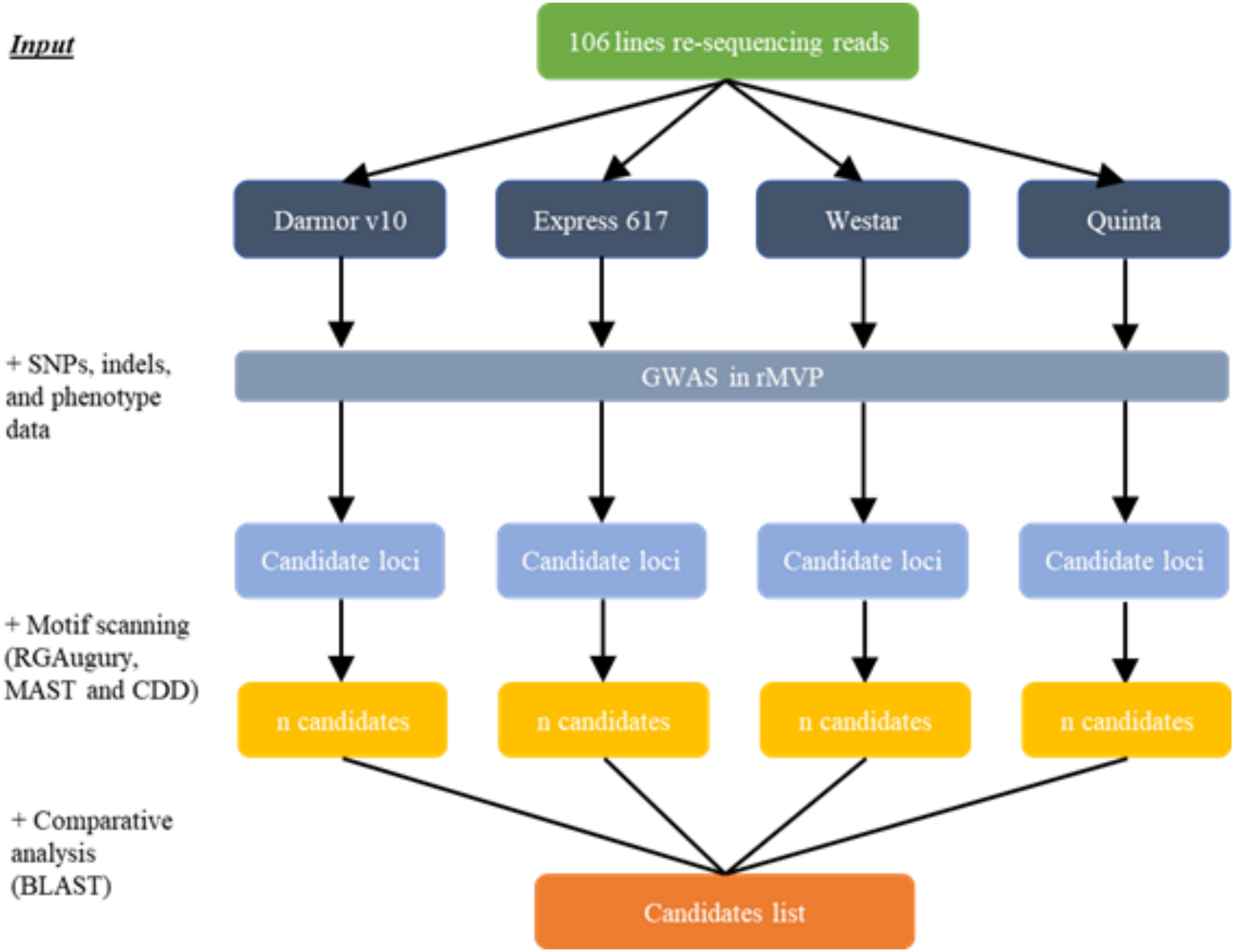
PCR primer were designed for the 16 candidates

- Initially mapped to 13.7 Mbp region, now narrowed to a 2.8 Mbp region
- 16 RGA candidates are being prioritised. PCR primers have been designed and will be used to amplify the candidates in a *LepR2*-resistant and *LepR2*-susceptible cultivar

LepR2 Candidates

Candidate	Chr	Size (bp)	Predicted gene type
cg_001	A10	3,065	RLK
cg_002	A10	3,104	RLK
cg_003	A10	2,752	RLK
cg_004	A10	2,646	RLK
cg_005	A10	3,521	RLK
cg_006	A10	2,918	TN
cg_007	A10	4,829	RLK
cg_008	A10	2,099	RLK
cg_009	A10	5,311	RLK
cg_010	A10	1,581	TM-CC
cg_011	A10	2,111	RLK
cg_012	A10	11,391	RLK
cg_013	A10	3,613	TM-CC
cg_014	A10	11,132	RLK
cg_015	A10	7,083	RLK
cg_016	A10	6,546	TM-CC

Analysis method – GWAS (*Rlm1, Rlm6-napus*)



Input

106 lines re-sequencing reads

Darmor v10

Express 617

Westar

Quinta

+ SNPs, indels, and phenotype data

GWAS in rMVP

Candidate loci

Candidate loci

Candidate loci

Candidate loci

+ Motif scanning (RGAugury, MAST and CDD)

n candidates

n candidates

n candidates

n candidates

+ Comparative analysis (BLAST)

Candidates list

SNPs and SVs used for GWAS

Genome	**Express 617**		**Quinta**		**Westar**	
Chromosome	**Number of SNPs**	**Number of SVs**	**Number of SNPs**	**Number of SVs**	**Number of SNPs**	**Number of SVs**
A01	149,608	19,334	122,071	15,871	122,560	16,039
A02	158,081	19,796	154,756	19,830	161,698	19,556
A03	177,753	26,081	179,628	26,047	172,814	24,026
A04	124,157	15,874	128,245	16,003	130,176	16,844
A05	141,195	17,847	145,804	18,343	155,643	18,266
A06	163,910	20,701	168,889	19,613	175,199	20,560
A07	158,786	20,885	143,869	18,784	160,348	20,517
A08	89,063	11,245	88,615	10,977	87,947	10,797
A09	198,691	23,804	224,630	25,644	213,419	23,040
A10	137,135	16,942	142,282	17,530	139,209	16,608
C01	245,419	23,195	244,704	22,047	245,593	22,446
C02	239,808	22,774	258,731	24,561	234,752	21,165
C03	284,126	33,081	282,493	32,899	258,159	30,355
C04	235,531	24,324	254,212	24,664	285,525	27,158
C05	100,708	11,616	99,511	11,039	95,941	10,744
C06	188,097	19,570	196,816	20,118	199,568	20,898
C07	166,259	18,661	218,264	21,773	209,971	20,362
C08	176,758	18,632	181,117	19,725	165,332	17,782
C09	131,716	14,105	122,467	12,807	109,649	11,527
Total A chromosomes	**1,498,379**	**192,509**	**1,498,789**	**188,642**	**1,519,013**	**186,253**
Total C chromosomes	**1,768,422**	**185,958**	**1,858,315**	**189,633**	**1,804,490**	**182,437**
Total (A+C)	**3,266,801**	**378,467**	**3,357,104**	**378,275**	**3,323,503**	**368,690**
	3,645,268		**3,735,379**		**3,692,193**	

Rlm1 candidates list (29)

(26 RLK, 1 RLP, 1 NBS, 1 TM-CC)

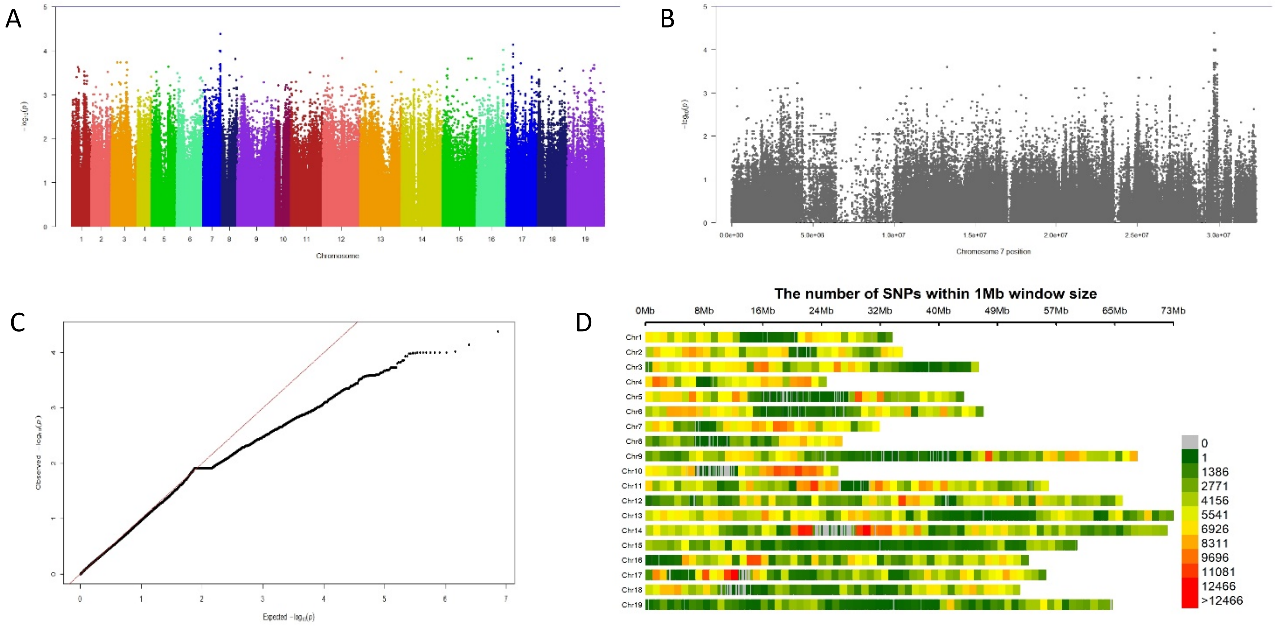
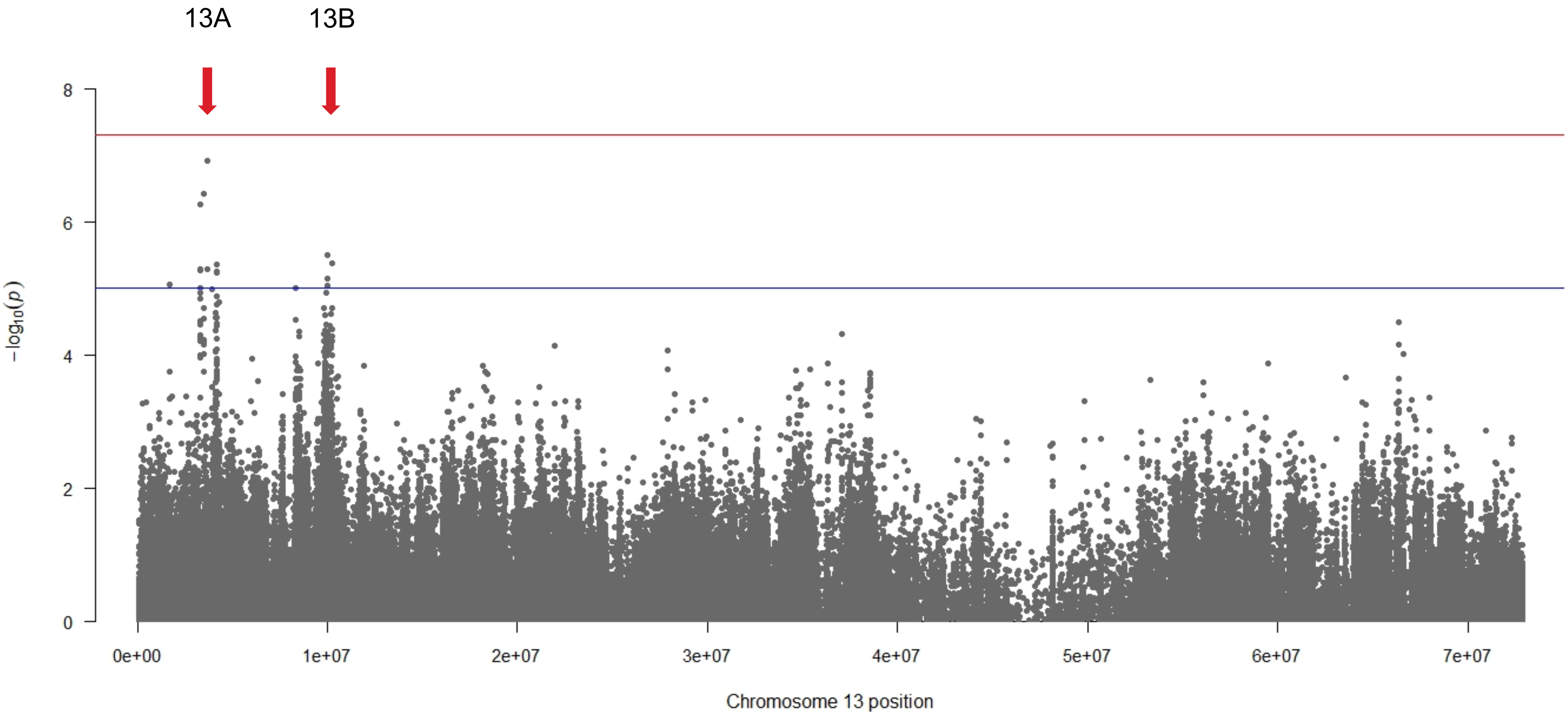


Figure 2. Manhattan plot for SNP association for *Rlm1* in Westar across the (A) whole-genome, (B) chromosome A07, and (C) QQ-plot using the 'qqman' package. The distribution of (D) SNP density across the whole genome as visualised using the 'rMVP' package.

Candidate	Length (bp)	Tool(s)	*R*-gene type
cd007	4.4 kb	RGA, MAST	RLK
cd007-21a	2.1 kb	MAST	RLK
Fu2019	2.8 kb	literature/MAST	STKc/RLK
cd007-21b	14.4 kb	MAST	RLK
cd007-21c	2.4 kb	MAST	RLK
cd008	2.0 kb	RGA, MAST	RLK
cd008-21a	1.6 kb	MAST	RLK
cd009	2.0 kb	RGA, MAST	RLK
cd010	2.0 kb	RGA, MAST	RLK
cd010-21a	2.4 kb	RGA	TM-CC
cd010b-21b	5.1 kb	MAST	RLK
cd010-21c	3.1 kb	RGA, MAST	RLK
cd010-21d	7.4 kb	RGA, MAST	RLK
cd010-21e	3.0 kb	RGA, MAST	RLK
cd010-21f	2.0 kb	MAST	RLK
cd011	7.3 kb	RGA	RLP
cd011-21a	1.3 kb	RGA	RLK
cd011-21b	2.6 kb	CDD	STKc/CDK9-like/RLK
cd012	4.0 kb	RGA, MAST	RLK
cd012-21a	4.7 kb	RGA, MAST	RLK
cd013	4.3 kb	RGA, MAST	RLK
cd014	1.7 kb	MAST	RLK
cd014-21a	3.2 kb	MAST	RLK
cd015	23.7 kb	RGA	TN
cd015-21a	8.0 kb	MAST	RLK
cd016	7.0 kb	RGA, MAST	RLK
cd016-21a	2.0 kb	MAST	RLK
cd017	8.6 kb	RGA, MAST	RLK
cd018	4.2 kb	RGA, MAST	RLK

Rlm6-napus candidate region

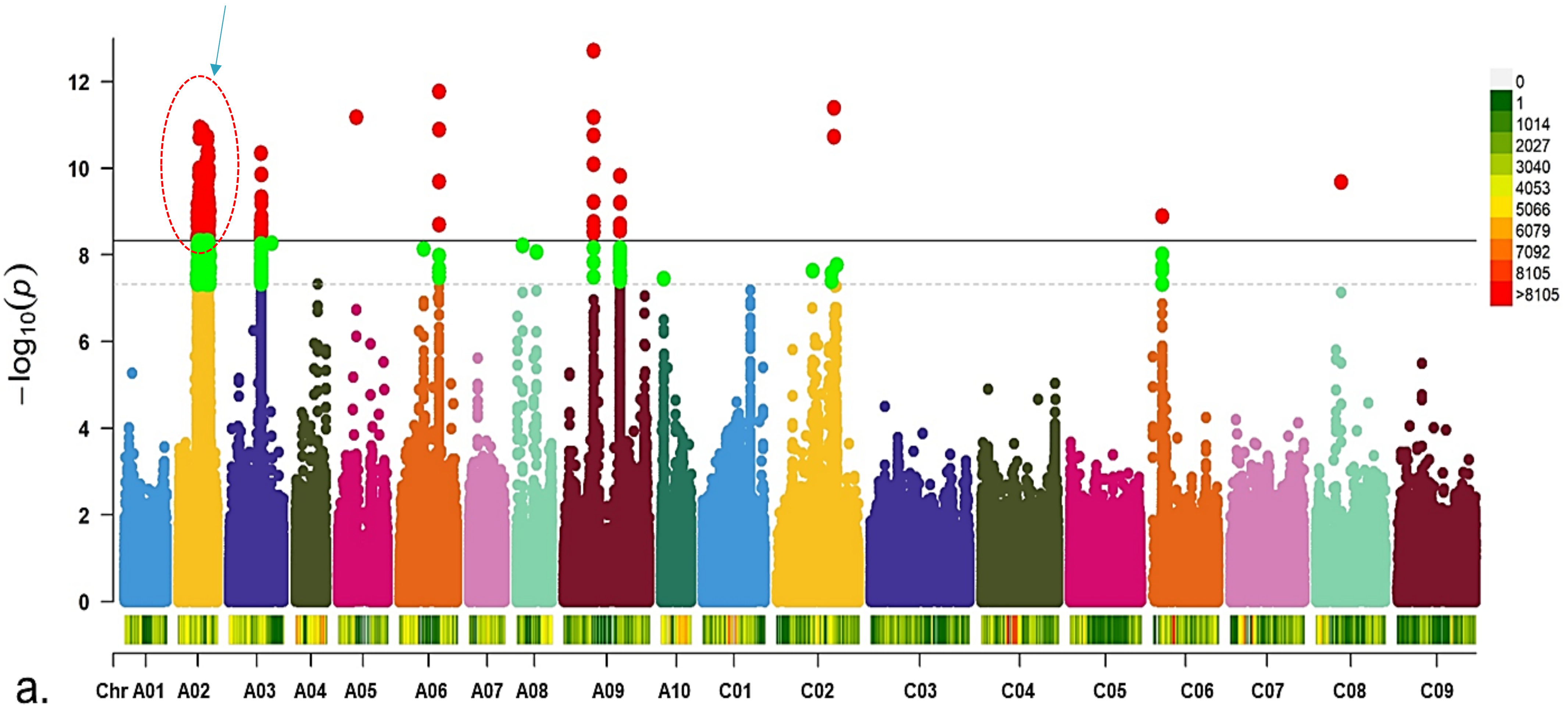


Rlm6-napus candidates list (44)

(33 RLK, 6 RLP, 2 NBS, 3 TM-CC)

Candidate	Length (bp)	Tool(s)	*R*-gene type
cd13aR6-001	2.4 kb	RGA, MAST	RLK
cd13aR6-002	9.8 kb	RGA, MAST	RLK
cd13aR6-003	23.3 kb	RGA, MAST	RLK
cd13aR6-004	4.5 kb	RGA, MAST	RLK
cd13aR6-005	9.9 kb	RGA, MAST	RLK
cd13aR6-006	3.2 kb	RGA, MAST	RLK
cd13aR6-007	125.4 kb	RGA, MAST	RLP
cd13aR6-008	4.2 kb	MAST	RLK
cd13aR6-009	14.1 kb	RGA, MAST	RLK
cd13aR6-010	4.4 kb	RGA, MAST	RLK
cd13aR6-011	2.3 kb	MAST	RLK
cd13aR6-012	0.9 kb	RGA	TM-CC
cd13aR6-013	5.9 kb	RGA, MAST	RLK
cd13aR6-014	2.6 kb	RGA, MAST	RLK
cd13aR6-015	14.1 kb	MAST	RLK
cd13aR6-016	4.3 kb	MAST	RLK
cd13aR6-017	3.7 kb	MAST	RLK
cd13aR6-018	1.6 kb	RGA	RLP
cd13aR6-019	7.4 kb	MAST	RLK
cd13aR6-020	10.7 kb	RGA, MAST	RLK
cd13aR6-021	2.2 kb	MAST	RLK
cd13aR6-022	1.3 kb	RGA	RLP
cd13aR6-023	2.0 kb	RGA	RLP
cd13aR6-024	7.4 kb	RGA	RLP
cd13aR6-025	5.2 kb	RGA	TM-CC

Candidate	Length (bp)	Tool(s)	*R*-gene type
cd13bR6-001	13.9 kb	RGA, MAST	TNL
cd13bR6-002	6.6 kb	RGA	TM-CC
cd13bR6-003	11.9 kb	MAST	RLP
cd13bR6-004	2.7 kb	MAST	RLK
cd13bR6-005	6.2 kb	MAST	RLK
cd13bR6-006	11.0 kb	RGA	TX
cd13bR6-007	14.6 kb	RGA, MAST	RLK
cd13bR6-008	4.6 kb	RGA, MAST	RLK
cd13bR6-009	5.4 kb	RGA	RLK
cd13bR6-010	2.0 kb	RGA, MAST	RLK
cd13bR6-011	2.3 kb	RGA, MAST	RLK
cd13bR6-012	2.1 kb	RGA, MAST	RLK
cd13bR6-013	3.9 kb	RGA	RLK
cd13bR6-014	7.3 kb	RGA, MAST	RLK
cd13bR6-015	10.1 kb	MAST	RLK
cd13bR6-016	2.4 kb	RGA, MAST	RLK
cd13bR6-017	4.7 kb	MAST	RLK
cd13bR6-018	5.0 kb	MAST	RLK
cd13bR6-019	3.5 kb	RGA	RLK



16 *LepR1* candidates (9 NLRs, 6 RLKs, & a TM-CC)

Manhattan plot (a) with SNP density below shows significant SNPs above the threshold line (green) in several chromosomes of *Brassica napus* Quinta genome.

SNP differences between resistant (n=24) and susceptible lines (n=43)

LepR1 candidate (SNP position=47)

	SNP	alleles	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	**R-T**	**S-T**	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	2	G/T	GT	GT	GG	GT	GT	GT	GG	GT	GT	GG	GG	GT	GT	GT	GT	GT	GG	GG	GG	GG	GG	TT	GT	GT	**GG**	**GT**	TT	GT	TT	TT	TT	GT	GT	GG	TT	GT	GT	GT	GT	GT	GT	TT	GT	GT	GT	TT	TT	TT	TT	TT	TT	TT	GG	TT	GT	GT	GT	GT	TT	GT	TT	TT	TT	GT	TT	TT	GT	GT	GT
CDS region	15	T/A	TA	TA	TA	TT	TA	TA	TA	TA	TA	TA	TA	AA	TA	TA	TA	TA	TA	TA	TA	TA	AA	TT	TT	TA	**TA**	**TT**	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT
CDS region	17	T/A	TA	TA	TA	TT	TA	TA	TA	TA	TA	TA	TA	AA	TA	TA	TA	TA	TA	TA	TA	TA	AA	TT	TT	TA	**TA**	**TT**	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT
CDS region	22	C/T	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CC	CT	CT	**CT**	**CC**	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC
	23	C/T	CC	CT	CT	CT	CT	CC	CT	CT	CC	CT	CT	CC	CC	CT	CC	CC	CC	CC	CT	CT	CC	CC	CT	CT	**CT**	**CC**	CC	CC	CC	CC	CC	CT	CT	CC	CC	CT	CT	CT	CC	CC	CC	CC	CT	CT	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CT	CT	CT	CT	CC	CC	CC	CC	CC	CT	CC	CC	CT	CT	CT
CDS region	25	A/T	AA	AT	AT	AA	AT	AT	AT	AT	AT	AA	AT	AA	AT	AT	AT	AT	AT	AT	AT	AT	AT	AA	AT	AT	**AT**	**AA**	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
	26	C/T	CC	CT	CT	CT	CT	CC	CT	CT	CC	CT	CT	CC	CC	CT	CC	CC	CC	CC	CT	CT	CC	CC	CT	CT	**CT**	**CC**	CC	CC	CC	CC	CC	CT	CT	CC	CC	CT	CT	CT	CC	CC	CC	CC	CT	CT	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CT	CT	CT	CT	CC	CT	CC	CC	CC	CT	CC	CC	CT	CT	CC
CDS region	27	G/A	GG	GA	GG	GG	GA	GA	GA	GA	GA	GG	GA	GG	GA	GA	GG	GA	GA	GA	GA	GA	GA	GG	GA	GA	**GA**	**GG**	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
	31	C/T	CT	CC	NN	CC	CC	CC	TT	CC	CT	CC	CC	CC	CC	CC	CT	CC	NN	CC	TT	TT	TT	CC	CC	CT	**TT**	**CC**	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC
	32	T/C	TC	TT	TT	TC	NN	TC	TT	TT	CC	CC	TT	TT	TT	TT	TT	TC	TT	TT	TT	TT	TT	TT	TC	TT	**TT**	**CC**	TT	CC	CC	CC	CC	CC	CC	CC	CC	TT	CC	CC	CC	CC	TT	CC	CC	CC	CC	NN	CC	CC	CC	CC	NN	NN	CC	CC	NN	TT	CC	CC	CC	CC	TT	CC	TT	CC	NN	CC	TT	CC	TT
	33	T/C	TC	NN	TT	TC	CC	TT	TT	TC	TT	TC	TT	TC	TC	TT	TC	TT	TC	TC	TT	TT	TT	CC	TC	TC	**TT**	**CC**	CC	CC	CC	TT	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	TT	CC	CC	CC	CC	CC	NN	NN	CC	NN	NN	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC
	34	A/G	AG	AA	AA	AG	NN	AG	AA	AG	NN	AG	AA	AG	AG	AG	AG	AA	AG	AG	AA	AA	AA	GG	AA	AA	**AA**	**GG**	GG	GG	NN	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	NN	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
	35	T/C	TC	TT	TT	TC	NN	TC	TT	TC	NN	TC	TT	TC	TC	TC	TC	TT	TC	TC	TT	TT	TT	CC	TT	TT	**TT**	**CC**	CC	CC	NN	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	NN	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC
	36	A/T	AT	NN	NN	AT	AA	AA	TT	AA	AA	TT	TT	AT	AT	AA	AT	AT	AA	AA	TT	TT	TT	AA	AA	AT	**TT**	**AA**	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	NN	AA	AA	AA	AA	NN	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
	37	A/G	AA	AA	NN	AG	AA	AA	AA	AG	AA	AG	AA	AG	AG	AG	AG	AA	AG	AG	AA	AA	AA	AA	AA	AA	**AA**	**GG**	GG	GG	AA	GG	GG	GG	GG	GG	GG	GG	GG	GG	AA	GG	GG	GG	GG	GG	GG	GG	AA	GG	GG	GG	GG	AA	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
	38	A/T	AT	AA	NN	AA	AA	AT	TT	AT	NN	AA	TT	AT	AT	AT	AT	AT	AT	AA	TT	TT	TT	AA	TT	AT	**TT**	**AA**	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
	39	A/C	AA	AA	AA	CC	AA	AA	AA	AC	NN	AC	AA	AC	AC	AA	AC	AA	AA	AA	AA	AA	AA	CC	AA	AA	**AA**	**CC**	CC	CC	AA	CC	CC	CC	CC	CC	CC	CC	CC	CC	AA	CC	CC	CC	CC	CC	AA	NN	AA	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC
	40	T/G	TT	TT	TT	TG	TT	TT	TT	TG	NN	GG	TT	TT	TG	TG	TT	TG	TT	TT	TT	TT	TT	GG	TT	TT	**TT**	**GG**	GG	GG	NN	GG	GG	GG	GG	GG	GG	GG	GG	GG	TT	GG	GG	GG	GG	TT	TT	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
	41	A/T	AA	AA	AA	AT	NN	AA	AA	AT	AA	TT	AA	AA	AT	AT	AT	AT	TT	AA	AA	AA	AA	TT	AA	AA	**AA**	**TT**	TT	TT	AA	TT	TT	TT	TT	TT	TT	TT	TT	TT	AA	TT	TT	TT	TT	TT	AA	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT
	42	G/T	GG	NN	GG	GT	TT	TT	GG	GG	GG	GT	GG	GG	GT	GT	GT	GT	TT	GG	GG	GG	GG	TT	GG	GG	**GG**	**TT**	TT	TT	TT	TT	TT	TT	GT	TT	TT	TT	TT	TT	GT	TT	TT	TT	TT	TT	GG	TT	TT	TT	TT	TT	TT	NN	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	GT	TT
	43	T/G	TT	TT	TT	GG	TT	TT	TT	TT	TT	TT	TT	TT	TG	TT	TG	TG	GG	TT	TT	TT	TT	GG	TT	TT	**TT**	**GG**	GG	GG	GG	GG	GG	GG	TT	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	TT	GG	GG	GG	GG	GG	GG	NN	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
	44	G/C	GC	GC	GG	GC	GG	GG	GG	GC	GG	GG	GG	GC	GG	CC	GC	GG	GG	GG	GG	GG	GG	CC	GC	GG	**GG**	**CC**	CC	CC	NN	CC	CC	CC	GG	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	NN	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC
	45	G/A	GA	GG	AA	GG	AA	GG	AA	GA	AA	GA	AA	GA	GG	GG	GA	GG	GA	GA	AA	AA	AA	GG	GA	GA	**AA**	**GG**	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
	46	G/A	GG	AA	GG	GA	GG	AA	GG	GA	NN	GA	GG	GA	AA	AA	GA	AA	NN	GA	GG	GG	GG	AA	GG	GG	**GG**	**AA**	AA	AA	NN	AA	AA	AA	AA	AA	GG	AA	AA	AA	AA	AA	NN	AA	AA	NN	AA	AA	AA	AA	AA	AA	AA	NN	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	GG	AA	AA	AA	AA

LepR1 candidate (SNP position=19)

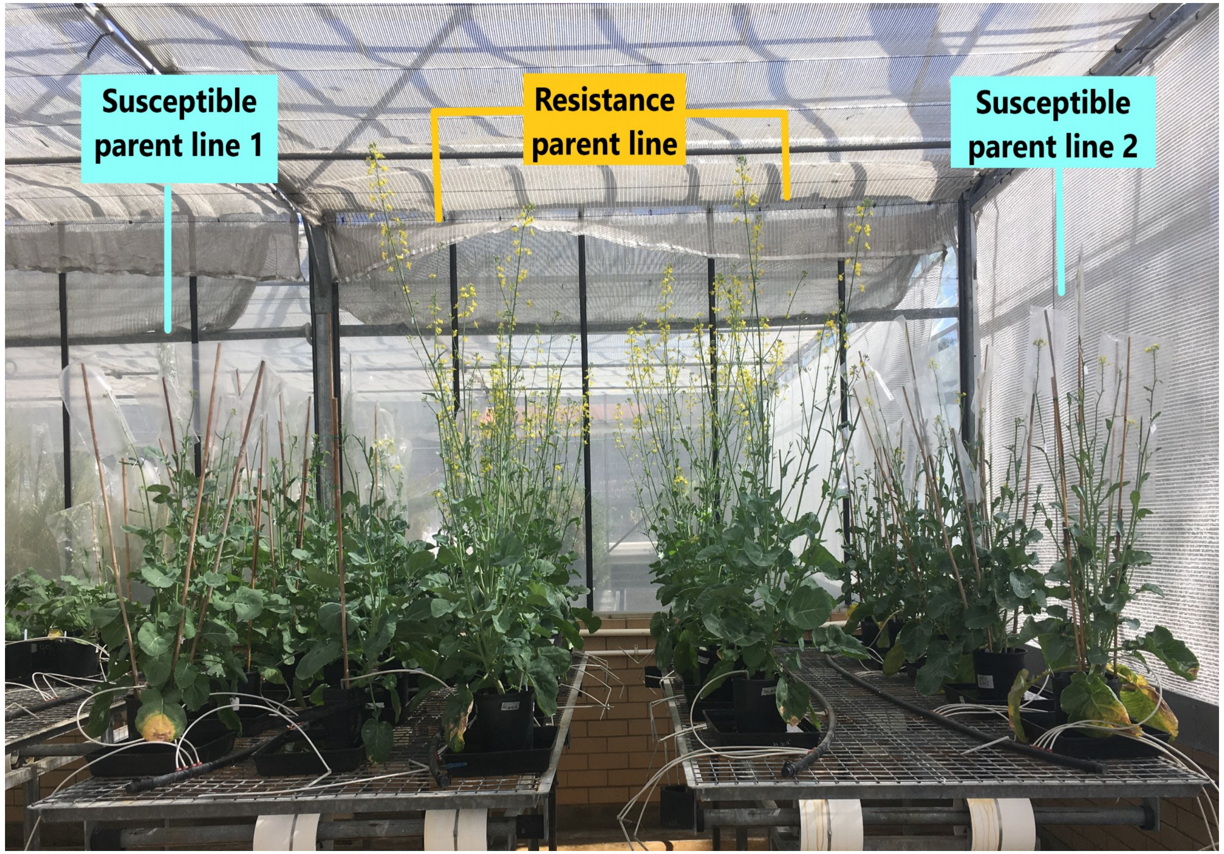
	SNP	alleles	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R-T	S-T	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	1	A/T	AA	AT	TT	AT	AT	AT	AT	AA	AT	AT	TT	AT	AA	AT	AA	AT	AT	AA	TT	TT	TT	AA	AT	AT	TT	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	NN	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
	2	A/G	AA	AG	GG	AG	AG	AG	AG	AA	AG	AG	GG	AG	AA	AG	AA	AG	AG	AA	GG	GG	GG	AA	AG	AG	GG	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	NN	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
	3	T/A	TA	TA	AA	TA	TA	TA	AA	TA	TA	TT	AA	TA	TA	TA	TA	AA	TT	TT	AA	AA	AA	TT	TA	TA	AA	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT
CDS region	4	C/G	CG	CG	GG	CG	CG	CG	GG	CC	CC	NN	GG	GG	CG	CG	CC	CG	CG	CG	GG	CG	GG	CC	CG	CC	GG	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC
CDS region	5	A/G	AA	AG	GG	AA	AG	AG	GG	GG	GG	AA	GG	AG	AG	AG	AA	GG	AA	AG	GG	GG	AA	AA	AA	AA	GG	GG	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
CDS region	6	T/C	TC	TC	CC	TT	TC	TC	CC	CC	TC	TT	CC	TC	TC	TC	TT	CC	TT	TC	CC	CC	TC	TT	TT	TT	CC	CC	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT
CDS region	7	G/A	GA	GA	AA	GG	GA	GA	AA	GA	GA	GA	AA	GA	GA	GA	GG	AA	GG	GA	AA	GA	GA	GG	GA	GA	AA	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
CDS region	8	G/A	GA	GA	AA	GG	GA	GA	AA	GA	GA	GG	AA	GA	GA	GA	GG	AA	GA	GA	AA	AA	AA	GG	GA	GA	AA	AA	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GA	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
CDS region	9	T/G	TG	TG	TG	TG	TG	TG	GG	TG	TG	TG	GG	TG	TG	TG	GG	GG	TG	TG	GG	GG	TG	TT	TG	TG	GG	GG	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TG	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT
CDS region	10	C/G	CG	CG	CG	CC	CG	CG	GG	CG	CG	CG	GG	CG	CG	CG	GG	GG	CG	CG	GG	GG	CG	CC	CG	CG	GG	GG	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CG	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC
CDS region	11	G/T	GT	GT	GT	GT	GT	GG	TT	GT	GT	GT	TT	GT	GT	GT	TT	GT	GG	GT	TT	TT	GT	GG	GT	GT	TT	TT	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GT	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
CDS region	12	A/G	AG	AG	AG	AG	AG	AG	GG	AG	AG	AG	GG	AG	AG	AG	GG	AG	AG	AG	GG	GG	AG	AA	AG	AG	GG	GG	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AG	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
CDS region	13	G/A	GA	GA	AA	GA	GA	GG	AA	GA	GA	AA	AA	GG	GA	GA	GA	GA	GG	GA	AA	AA	AA	GG	GA	GA	AA	AA	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GA	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
	14	C/T	CC	CC	TT	CC	CC	CC	TT	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	TT	TT	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC
	15	G/A	GG	GG	AA	GG	GG	GG	AA	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	AA	GA	GG	GG	GG	GG	GG	AA	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GA	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
	16	C/G	CC	CC	GG	CC	CC	CC	GG	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	GG	CG	CC	CC	CC	CC	CC	GG	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CG	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC
	17	A/C	AA	NN	NN	AA	AA	AA	NN	AA	AA	AC	CC	AA	AA	AA	AA	AA	AA	AA	CC	CC	CC	AA	AA	AA	NN	NN	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AC	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
	18	T/C	TT	TC	CC	TC	TC	TT	CC	TC	CC	TC	CC	TC	TT	TT	TC	TC	TC	TC	CC	CC	CC	TT	TT	TT	CC	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT
	19	A/T	AA	AT	TT	AT	AT	AA	TT	AT	TT	AT	TT	AT	AA	AT	AT	AT	AT	AT	TT	TT	TT	AA	AA	AA	TT	TT	AA	AA	AA	AA	AA	AA	NN	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AT	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA

LepR1 candidate (SNP position=9)

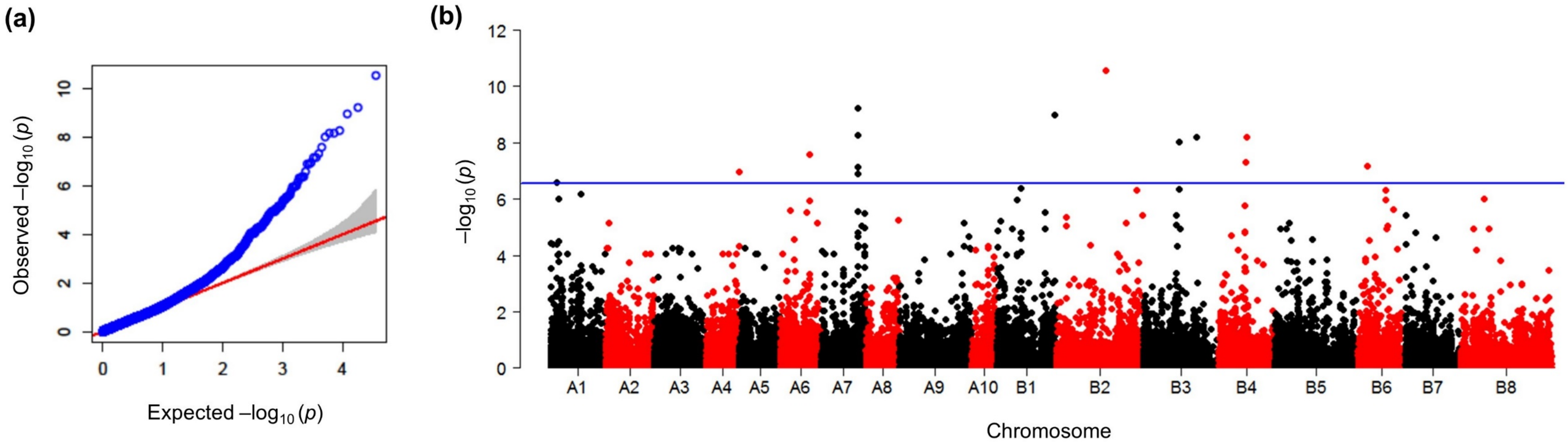
	SNP	alleles	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R-T	S-T	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
CDS region	1	C/G	CG	CG	CG	CC	CG	CG	GG	CG	CC	CG	CG	CC	CG		CG	CC	CG	CG	GG	GG	GG		CG	CG	GG	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC
	2	T/C	TC	TC	CC	TT	CC	TT	CC	TC	TT	TC	CC	TT	TC	TT	TC	TT	TC	TT	CC	CC	CC	TT	TT	TC	CC	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT
CDS region	3	A/T	AT	AA	TT	AA	TT	AA	TT	AT	AA	AA	AA	AT	AT	AT	AT	AT	AT	AA	TT	AT	TT	AA	AA	AA	TT	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
	4	A/C	AA	AA	CC	AA	AA	AA	CC	AC	AC	CC	CC	AA	AC	AC	AA	AA	AC	AC	CC	CC	CC	AA	AA	AA	CC	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	NN	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
	5	G/A	GA	GA	AA	GA	GA	GG	AA	GA	GA	AA	AA	GG	GA	GA	GA	GA	GA	GA	AA	AA	AA	GG	GA	GG	AA	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
	6	T/G	TG	TG	GG	TG	TG	TG	GG	TG	TG	GG	GG	TT	TG	TG	TG	TT	TG	TG	GG	GG	GG	TT	TG	TG	GG	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT
	7	A/T	AT	AA	TT	AT	AT	AT	TT	AT	AT	TT	TT	AA	AT	AT	AT	AT	AT	AT	TT	TT	TT	AA	AT	AT	TT	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
	8	G/A	GA	GA	AA	GA	GA	GG	AA	GA	GA	GA	AA	GA	GA	GA	GA	GA	GG	GG	AA	GA	AA	GG	GG	GG	AA	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	NN	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
	9	A/G	AG	AG	GG	AG	AG	AG	GG	AG	AA	GG	GG	AA	AG	GG	AG	AG	AA	AA	GG	GG	GG	AA	AG	AA	GG	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA

RlmS resistance X *rlmS* susceptible F1 population

Use cross-pollination to produce F1 generation



Rlm6-juncea

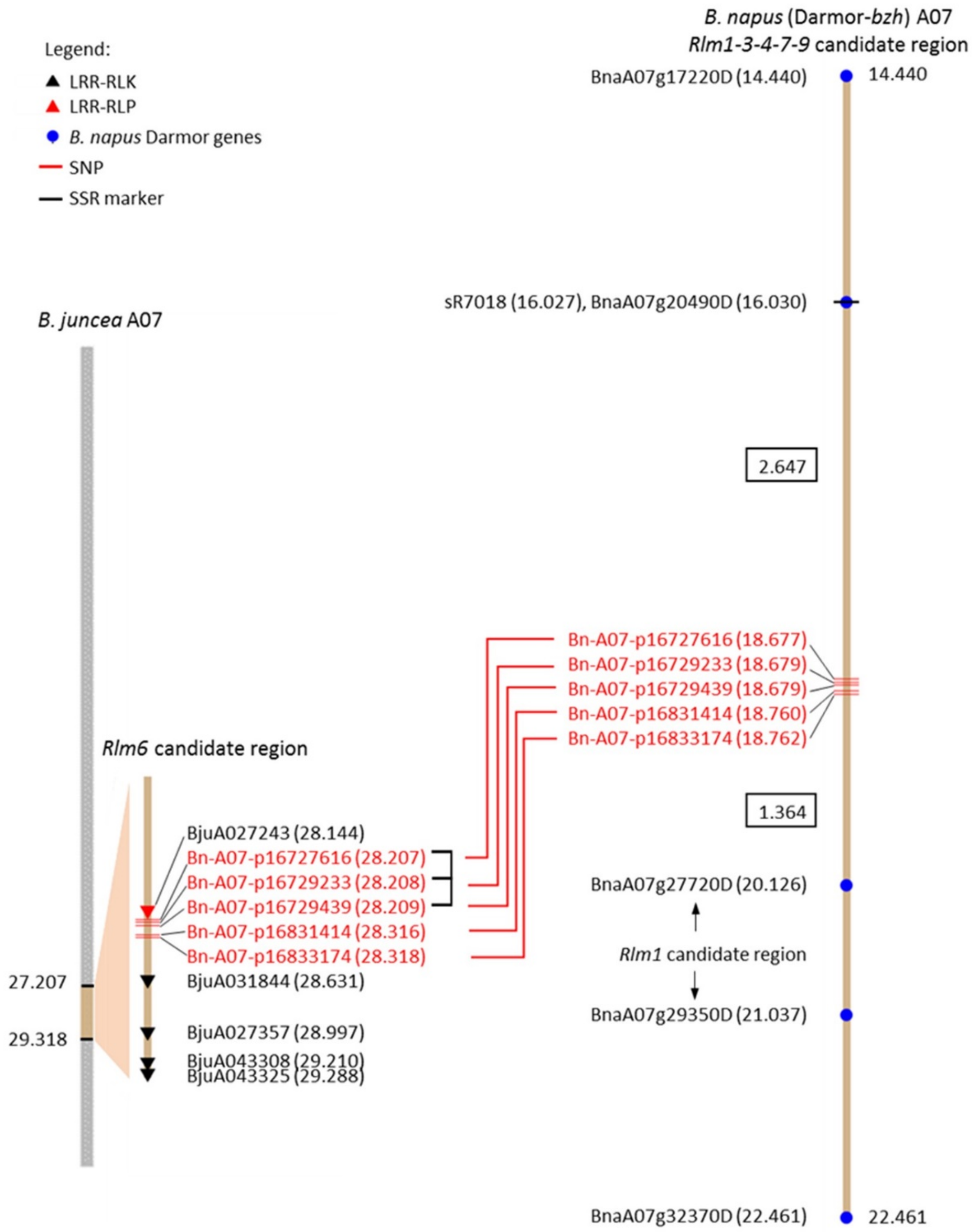

(a)
Observed –log10 (p)
0
2
4
6
8
10
0
1
2
3
4
Expected –log10 (p)
(b)
–log10 (p)
0
2
4
6
8
10
12
A1
A2
A3
A4
A5
A6
A7
A8
A9
A10
B1
B2
B3
B4
B5
B6
B7
B8
Chromosome

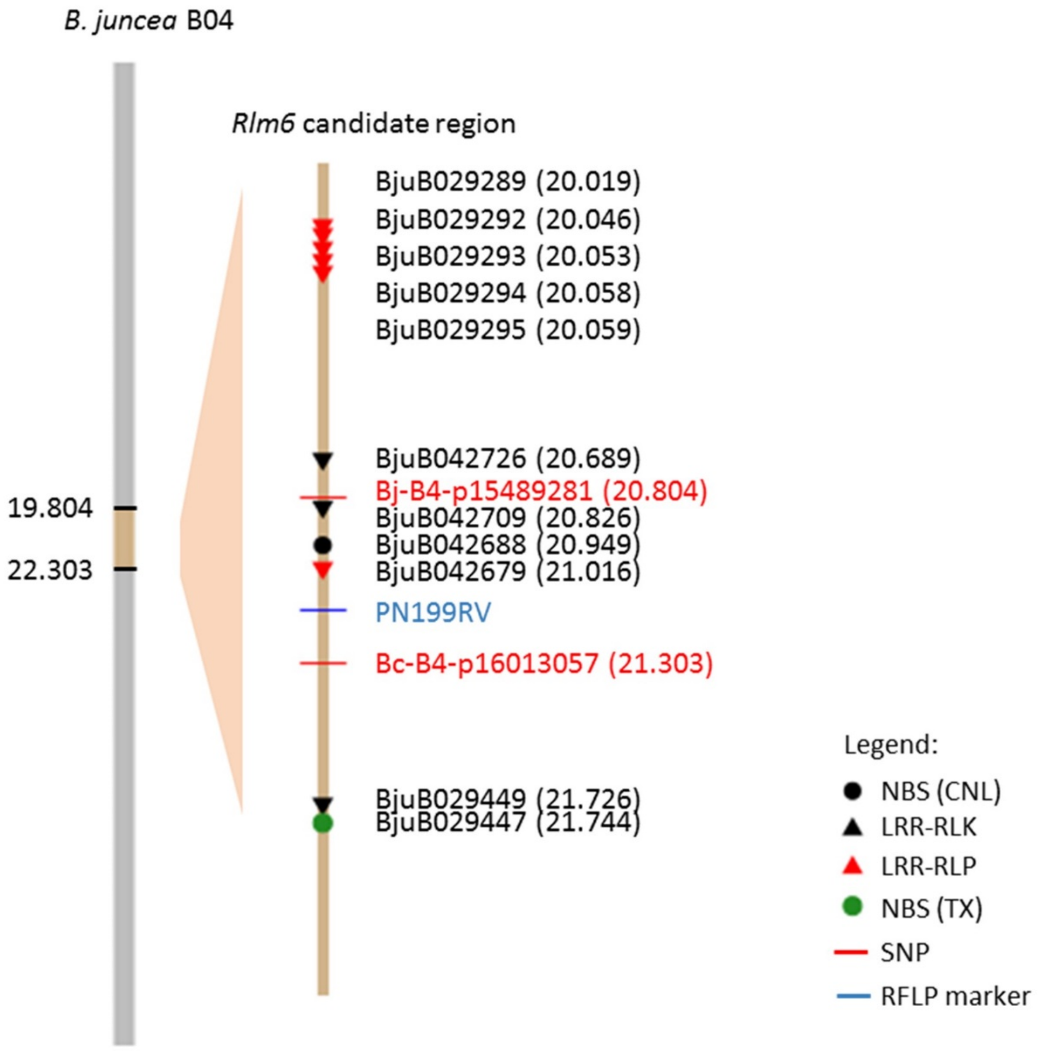
Blast results of significant SNPs obtained through GWAS in *B. juncea* v1.5 genome

SNP	**Chromosome**	**Hit start**	**Hit end**	**E value**	**Identity (%)**	**Query Coverage (%)**
Bn-A01-p4113202	A01	4,611,171	4,611,220	1.00E-10	92.00	100.00
	B05	6,151,185	6,151,234	3.00E-08	90.00	100.00
Bn-A03-p6385958	A03	8,011,002	8,011,051	3.00E-20	100.00	100.00
	A04	25,425,014	25,425,063	3.00E-20	100.00	100.00
Bn-A06-p17938497	A06	22,395,971	22,396,020	3.00E-20	100.00	100.00
Bn-A07-p16727616	A07	28,207,331	28,207,380	6.35E-18	100.00	100.00
Bn-A07-p16729233	A07	28,208,895	28,208,944	6.35E-18	100.00	100.00
Bn-A07-p16729439	A07	28,209,101	28,209,150	2.70E-16	98.00	100.00
Bn-A07-p16831414	A07	28,316,510	28,316,560	9.43E-16	98.00	100.00
Bn-A07-p16833174	A07	28,318,268	28,318,317	6.35E-18	100.00	100.00
Bc-B1-p39434162	B01	44,284,882	44,285,000	1.00E-46	95.00	99.17
	B01	44,273,318	44,273,436	1.00E-46	95.00	99.17
	A05	30,245,924	30,246,041	1.00E-32	90.00	99.17
Bc-B2-p28312260	B02	40,388,605	40,388,727	1.00E-52	95.90	100.00
	B02	38,236,943	38,237,065	1.00E-52	95.90	100.00
Bc-B3-p30806193	B03	41,403,586	41,403,706	2.00E-60	98.30	100.00
	A07	33,269,094	33,269,208	1.00E-35	90.10	100.00
Bj-B3-p21937305	B03	27,981,715	27,981,843	1.60E-32	83.70	100.00
	B03	27,859,809	27,859,929	1.00E-59	97.50	100.00
	A07	28,152,803	28,152,930	5.59E-32	82.80	100.00
Bc-B4-p16013057	B04	21,325,733	21,325,852	7.00E-62	100.00	99.17
	B04	21,303,022	21,303,141	7.00E-62	100.00	99.17
Bj-B4-p15489281	B04	20,804,276	20,804,396	1.00E-59	99.20	100.00
Bc-B6-p7694203	B06	7,549,193	7,549,313	4.00E-45	91.70	100.00
	A06	18,618,264	18,618,383	4.00E-29	88.50	100.00

Table 4 Location of previously reported markers linked with *Rlm6* gene

Gene	Type	Primer	Chromosome	Hit start	Hit end	Sequence length (bp)	Reference
Rlm6	SCAR	B5Rlm6_1_F	B01	17,531,557	17,531,534	24	Rashid et al. (2018)
		B5Rlm6_1_R	B01	17,530,912	17,530,934	23	
	CAPS	BjHZ_1_F	A07	9,434,730	9,434,749	20	
		BjHZ_1_R	A07	9,435,258	9,435,239	20	
		BnHZ_2_F	A07	9,434,906	9,434,929	24	
		BnHZ_2_R	A07	19,448,560	19,448,578	19	
		BjHZ_1_F	A09	4,777,090	4,777,109	20	
		BjHZ_1_R	A09	4,777,618	4,777,599	20	
		BnHZ_2_F	A09	4,777,266	4,777,289	24	
		BnHZ_2_R	A09	4,777,830	4,777,807	24	
	RAPD	OPT01-800	NA	NA	NA	NA	Chèvre et al. (1997)
		OPI01-HaeIII	NA	NA	NA	10	
		OPG02-800	NA	NA	NA	10	
LMJR1	RFLP	PN199RV	B04	21,141,546	21,142,653	1113	Christianson et al. (2006)
	SSR	sB31143F	NA	NA	NA	NA	
	SSR	sB1936	NA	NA	NA	NA	


Legend:
▲ LRR-RLK
🔺 LRR-RLP
● B. napus Darmor genes
SNP
SSR marker
B. napus (Darmor-bzh) A07
Rlm1-3-4-7-9 candidate region
BnaA07g17220D (14.440)
14.440
sR7018 (16.027), BnaA07g20490D (16.030)
2.647
Bn-A07-p16727616 (18.677)
Bn-A07-p16729233 (18.679)
Bn-A07-p16729439 (18.679)
Bn-A07-p16831414 (18.760)
Bn-A07-p16833174 (18.762)
1.364
BnaA07g27720D (20.126)
Rlm1 candidate region
BnaA07g29350D (21.037)
BnaA07g32370D (22.461)
22.461
B. juncea A07
Rlm6 candidate region
BjuA027243 (28.144)
Bn-A07-p16727616 (28.207)
Bn-A07-p16729233 (28.208)
Bn-A07-p16729439 (28.209)
Bn-A07-p16831414 (28.316)
Bn-A07-p16833174 (28.318)
27.207
BjuA031844 (28.631)
29.318
BjuA027357 (28.997)
BjuA043308 (29.210)
BjuA043325 (29.288)


B. juncea B04
19.804
22.303
Rlm6 candidate region
BjuB029289 (20.019)
BjuB029292 (20.046)
BjuB029293 (20.053)
BjuB029294 (20.058)
BjuB029295 (20.059)
BjuB042726 (20.689)
Bj-B4-p15489281 (20.804)
BjuB042709 (20.826)
BjuB042688 (20.949)
BjuB042679 (21.016)
PN199RV
Bc-B4-p16013057 (21.303)
BjuB029449 (21.726)
BjuB029447 (21.744)
Legend:
● NBS (CNL)
▲ LRR-RLK
▲ LRR-RLP
● NBS (TX)
— SNP
— RFLP marker

Summary of candidate genes located in the potential regions of 1 Mb upstream and downstream of the associated SNPs

Chromosome	Resistance gene				Non-resistance gene			Total
	NLR	LRR-RLK	LRR-RLP	Total	RLK-other receptor	Others	Total	
A07	0	4	1	5	6	272	278	283
B04	2	3	6	11	2	289	291	302
Total	2	7	7	16	8	561	569	585

Characterisation and genotypes of candidate *Rlm6* genes identified in the GWAS regions on chromosomes A07 and B04 in *B. juncea*

Chromosome	Gene ID	Position		RGAugury annotation	SNP position	B. juncea varieties				Alleles
		Start	End			Rlm6 (Resistant)		rlm6 (Susceptible)		
						XinYouNo.9	Yubileynaya	Lavia	ATC93623	
A07	BjuA027243	28,144,420	28,146,546	RLP-LRR	-	-	-	-	-	-
	BjuA031844	28,631,629	28,634,613	RLK-LRR	-	-	-	-	-	-
	BjuA027357	28,997,793	29,000,166	RLK-LRR	28,998,036	G	×	×	×	G/C
					28,998,087	C	×	×	×	C/T
					28,998,253	T	×	T	×	T/G
					28,999,589	G	×	×	T	G/T
					28,999,671	G	A	×	A	G/A
	BjuA043308	29,210,208	29,212,960	RLK-LRR	29,210,850	×	T	×	T	T/C
					29,210,899	×	G	×	G	G/T
					29,211,109	×	×	×	×	C/A
					29,211,515	×	A	×	A	A/G
					29,212,050	×	G	G	G	G/A
					29,212,051	×	T	T	T	T/C
					29,212,433	A	×	×	T	T/A
					29,212,498	G	×	×	T	T/G
					29,212,681	A	A	G	G	G/A
	BjuA043325	29,288,182	29,292,161	RLK-LRR	-	-	-	-	-	-

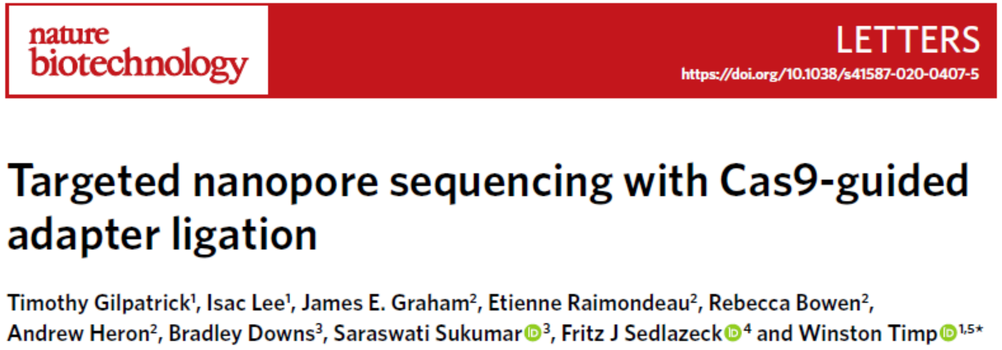
Chromosome	Gene ID	Position		RGAugury annotation	SNP position	B. juncea varieties				Alleles
		Start	End			Rlm6 (Resistant)		rlm6 (Susceptible)		
						XinYouNo.9	Yubileynaya	Lavia	ATC93623	
B04	BjuB029289	20,019,456	20,021,741	RLP-LRR	20,019,576	×	G	×	A	A\G
					20,020,473	×	×	×	C	C\T
					20,020,492	×	×	×	C	C\T
					20,020,513	×	×	×	C	C\G
					20,020,719	×	G	×	A	A\G
					20,020,760	×	A	×	T	T\A
					20,020,876	A	×	×	×	G\A
					20,020,898	G	×	×	T	T\G
					20,020,962	×	G	×	C	C\G
					20,021,177	G	G	G	T	T\G
					20,021,196	A	A	A	T	T\A
	BjuB029292	20,045,795	20,047,543	RLP-LRR	-	-	-	-	-	-
	BjuB029293	20,053,332	20,055,551	RLP-LRR	-	-	-	-	-	-
	BjuB029294	20,057,677	20,059,125	RLP-LRR	20,058,043	C	×	×	×	C\A
					20,058,329	×	G	×	A	A\G
					20,058,343	×	×	×	C	C\T
					20,058,368	×	×	×	G	G\T
					20,058,373	×	×	×	T	T\C
	BjuB029295	20,059,248	20,059,790	RLP-LRR	-	-	-	-	-	-
	BjuB042726	20,688,854	20,697,838	RLK-LRR	20,694,050	×	G	×	A	A\G
					20,694,052	×	G	×	A	A\G
					20,694,802	T	T	×	C	C\T
					20,694,862	T	T	×	C	C\T
					20,694,880	A	A	×	G	G\A
					20,694,986	T	×	×	C	C\T
					20,694,997	G	×	×	T	T\G
					20,695,213	G	G	×	A	A\G
					20,695,396	×	×	×	G	G\A
					20,695,402	×	×	×	G	G\A
					20,697,755	×	×	×	A	A\G

Chromosome	Gene ID	Position		RGAugury annotation	SNP position	B. juncea varieties				Alleles
		Start	End			Rlm6 (Resistant)		rlm6 (Susceptible)		
						XinYouNo.9	Yubileynaya	Lavia	ATC93623	
B04	BjuB042709	20,825,888	20,828,011	RLK-LRR	20,825,957	×	×	×	×	G\A
					20,826,194	×	A	×	G	G\A
					20,826,215	×	A	×	G	G\A
					20,826,314	×	×	×	×	A\G
					20,826,341	×	×	×	×	G\C
					20,826,437	T	×	T	×	A\T
					20,826,500	G	G	G	A	A\G
					20,826,654	×	×	×	×	C\G
					20,826,660	×	×	×	×	G\T
					20,826,785	C	C	×	A	A\C
					20,826,875	T	T	T	G	G\T
					20,827,001	T	×	×	×	G\T
					20,827,232	C	T	×	T	T\C
					20,827,832	G	G	G	A	G\A
	BjuB042688	20,948,609	20,953,450	NLR (CNL)	20,950,081	A	A	A	A	A\C
					20,951,169	G	×	G	T	T\G
					20,951,191	C	×	C	T	T\C
					20,952,479	×	T	×	×	T\A
	BjuB042679	21,016,359	21,019,760	RLP-LRR	21,018,981	C	C	×	×	C\T
					21,019,422	G	×	×	T	G\T
					21,019,746	×	×	A	A	A\T
	BjuB029449	21,726,142	21,731,226	RLK-LRR	21,726,449	G	G	×	C	G\C
					21,726,470	A	A	×	×	A\C
					21,726,598	A	A	×	×	A\C
					21,726,627	G	G	×	×	G\T
					21,727,167	C	C	×	T	C\T
					21,727,174	C	C	×	A	C\A
					21,727,235	C	C	×	×	C\T
					21,727,682	G	G	×	T	G\T
					21,727,696	C	C	T	T	C\T
					21,727,972	A	A	×	C	A\C
					21,728,334	×	A	C	×	A\C
					21,728,780	C	C	A	×	C\A
					21,728,910	T	T	×	×	T\G
					21,728,927	G	G	×	×	G\A
					21,728,959	T	T	×	C	T\C
					21,729,160	A	A	×	T	A\T
					21,729,235	A	×	×	G	A\G
					21,729,743	A	A	T	×	A\T
					21,729,744	C	C	A	×	C\A
					21,729,952	T	C	T	T	T\C
					21,730,107	×	×	×	A	T\A
					21,731,073	T	×	×	C	T\C
	BjuB029447	21,744,102	21,747,180	NLR (TX[a])	21,744,657	A	A	T	×	A\T
					21,744,963	×	G	G	×	G\T
					21,745,130	×	A	×	G	A\G
					21,745,415	G	G	T	×	G\T
					21,745,717	T	T	G	G	T\G
					21,746,187	A	A	A	A	A\T
					21,746,423	C	C	×	T	C\T
					21,746,622	G	×	×	A	G\A

Nanopore Cas9-targeted sequencing (nCATS)

- Targeted sequencing approach coupled with long read nanopore sequencing
- Proven to be a cheap and fast method to obtain high coverage reads of the gene/region of interest

- Up to 78 kb achieved in human cells with over 650X sequencing coverage



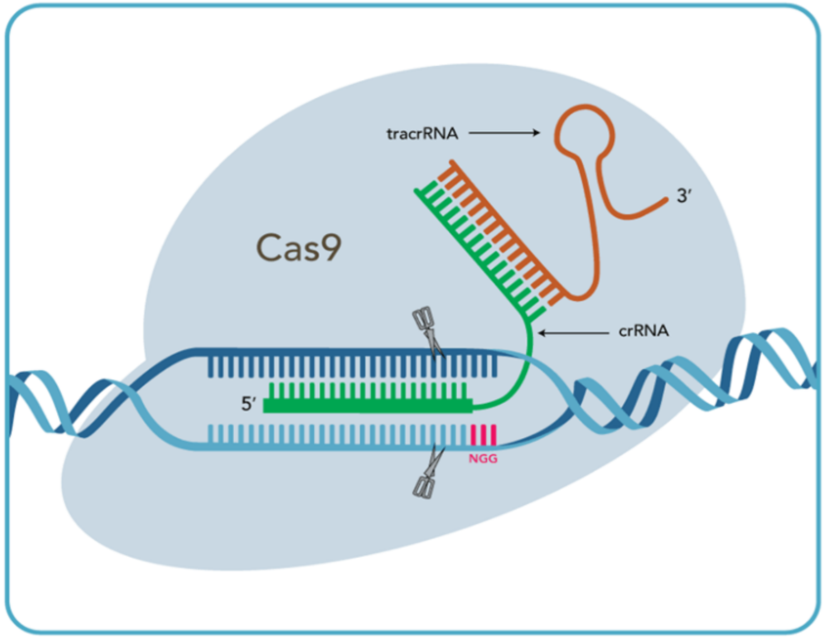
nature biotechnology

LETTERS

https://doi.org/10.1038/s41587-020-0407-5

Targeted nanopore sequencing with Cas9-guided adapter ligation

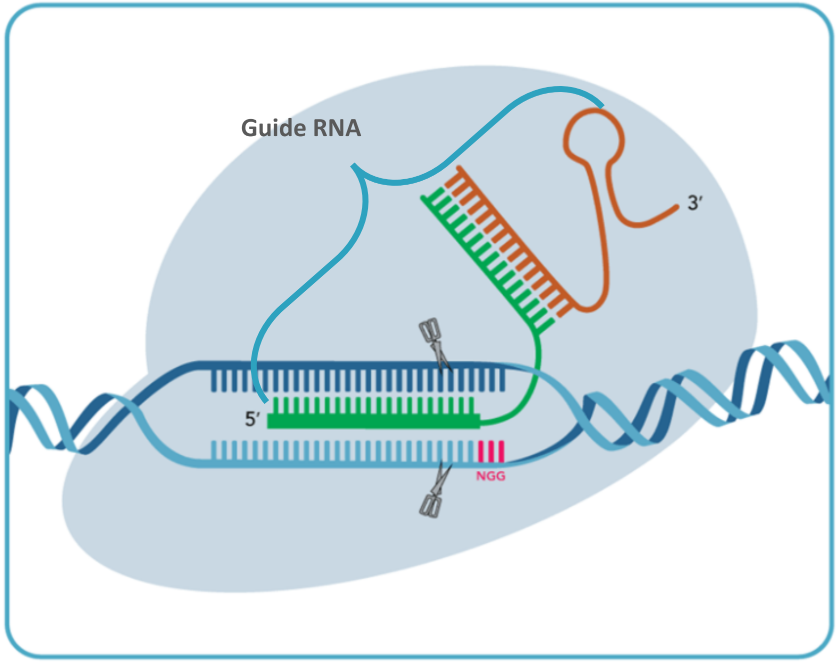
Timothy Gilpatrick[1], Isac Lee[1], James E. Graham[2], Etienne Raimondeau[2], Rebecca Bowen[2],
Andrew Heron[2], Bradley Downs[3], Saraswati Sukumar[3], Fritz J Sedlazeck[4] and Winston Timp[1,5]*



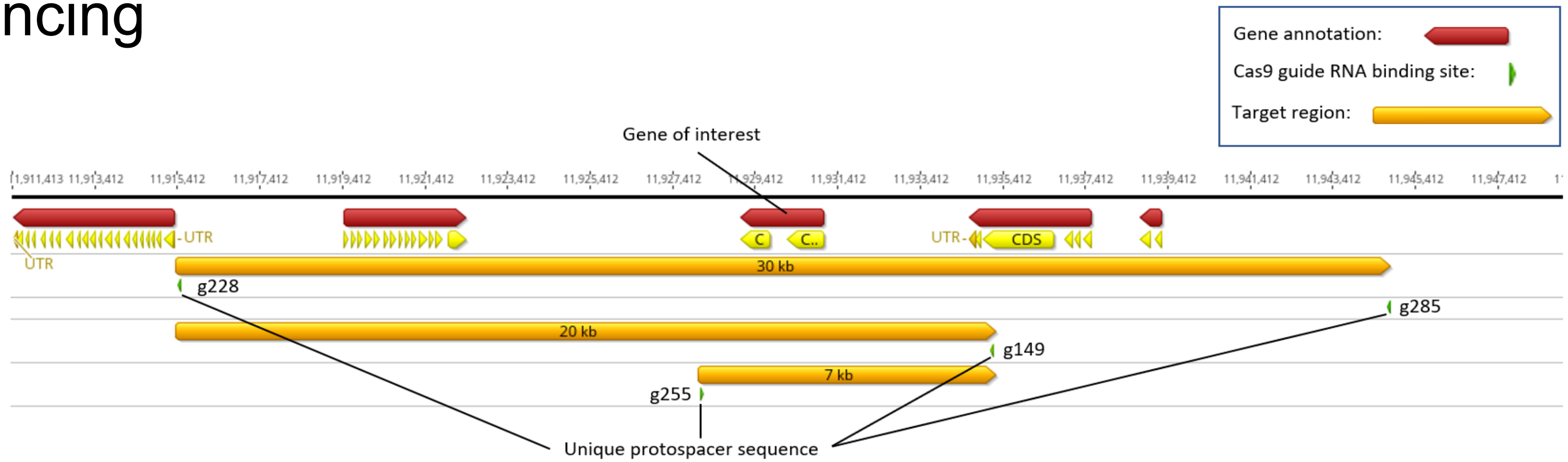
nCATS in canola

- Guide RNA for the target regions that contain the gene of interest (7kb, 20kb, 30kb) have been designed

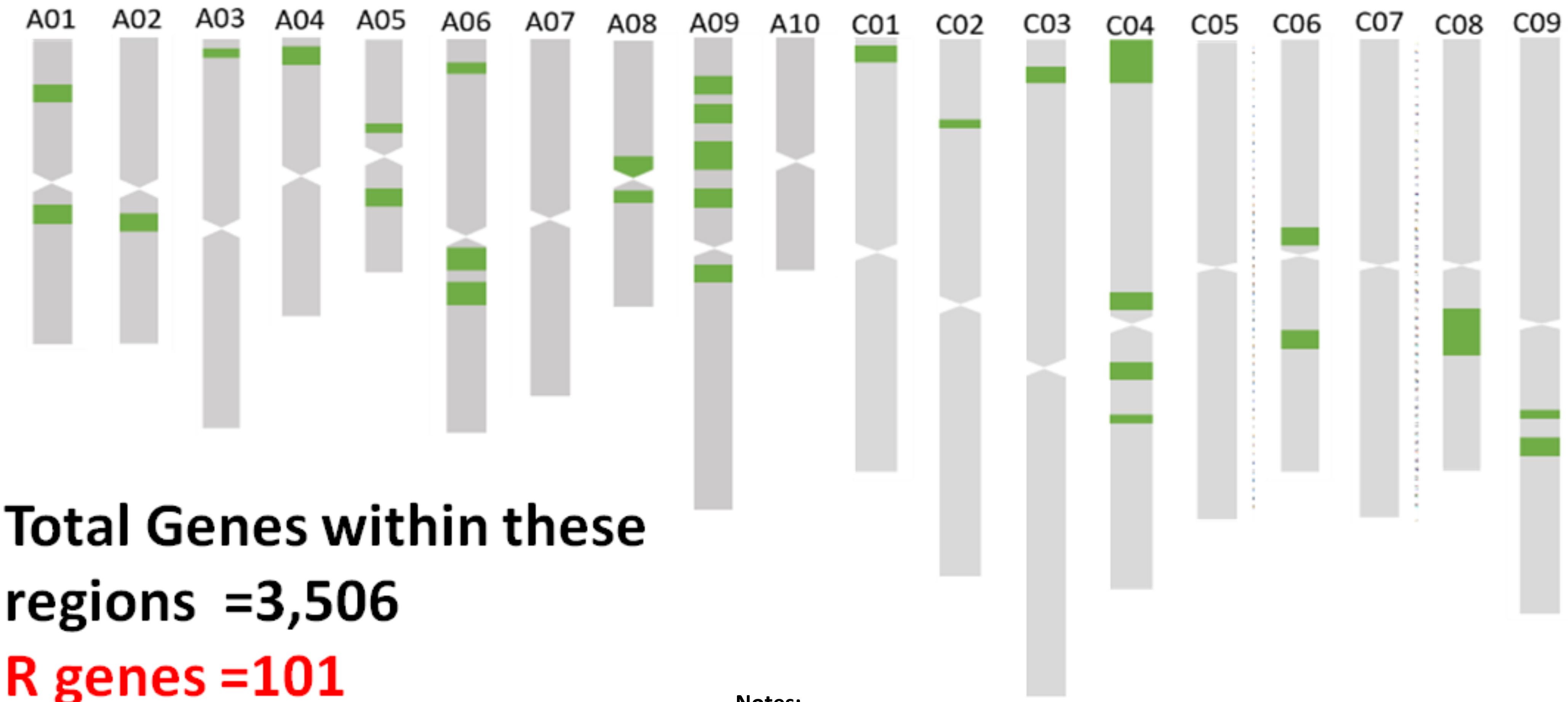
- Validating Cas9 specificity and efficiency using PCR and Sanger sequencing



Modified from IDT (https://sg.idtdna.com/pages/products/crispr-genome-editing)



Meta-analysis found 36 consistent genomic regions for QR



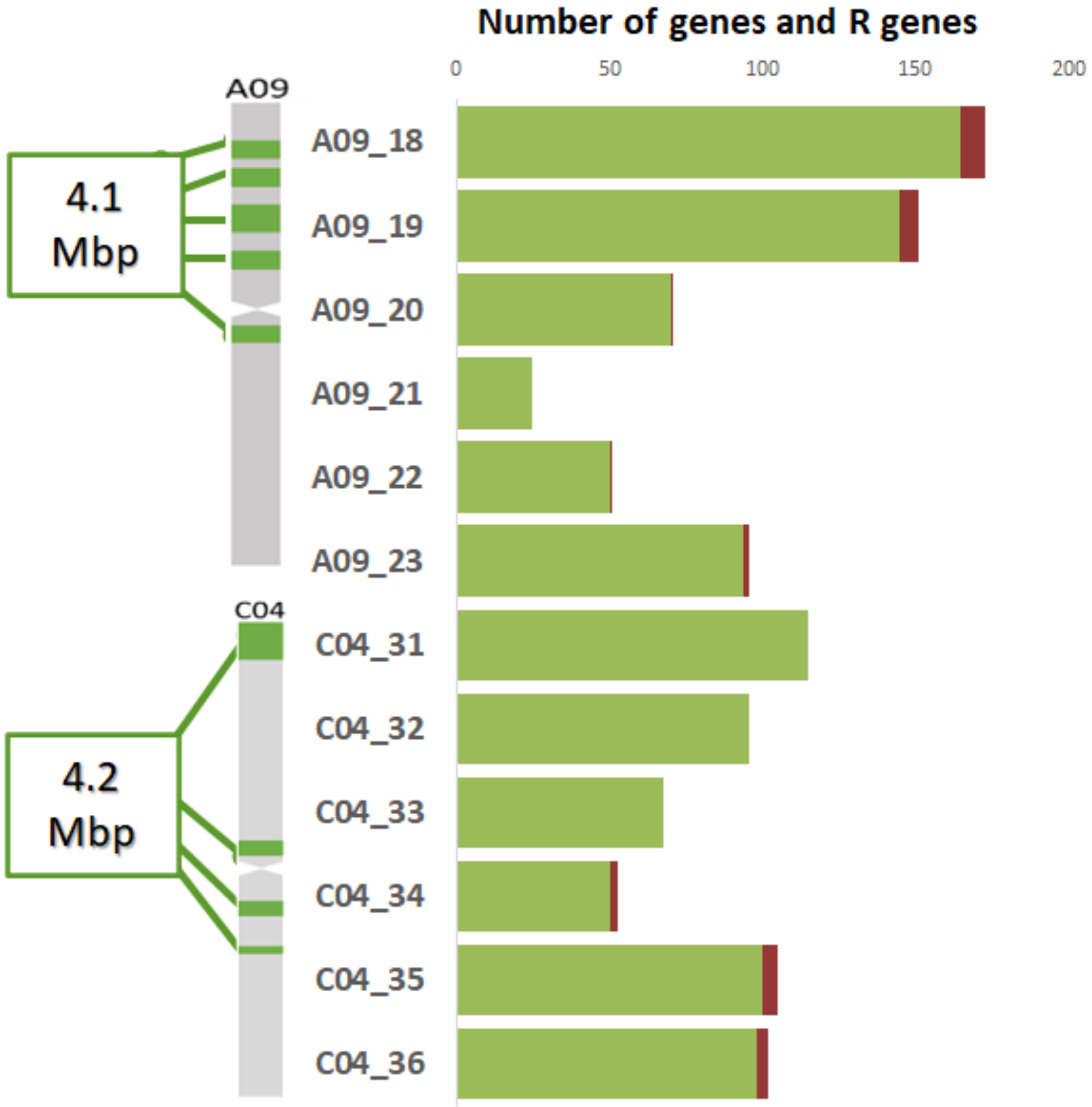
Total Genes within these regions =3,506

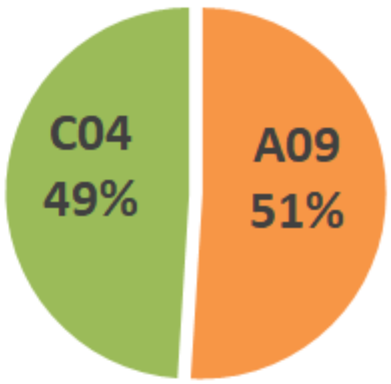
R genes =101

Notes:

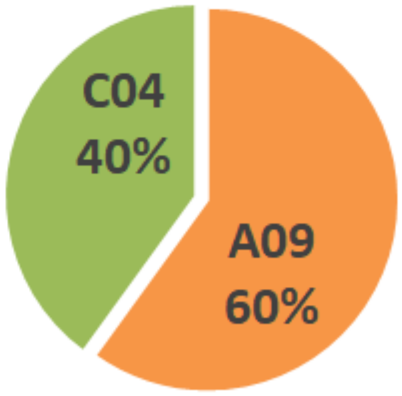
- Meta-analysis identified 36 consistent genomic regions for quantitative resistance distributed in the A and C genomes across chromosomes. Between the genomes, the A genome has slightly higher number of consistent regions compared with the C genomes. But in terms of the number of genes, they contained more or less equal number of genes.
- We excluded A07 and A10, since these are where most of the R-genes were mapped.

A09 and C04 are genomic "hot-spots" for quantitative resistance





Proportion of genes within A09 and C04



Proportion of R genes within A09 and C04

List of *LepR3* homolog RGAs per species (>70% similarity)

Gene	Org	Closest Genomic Feature	Rga main	Rga subclass
Bna_LepR3	Arabidopsis thaliana (TAIR10)	AT2G15042.1	RLP	lrr
Bna_LepR3	Arabidopsis thaliana (TAIR10)	AT3G05650.1	RLP	lrr
Bna_LepR3	Arabidopsis thaliana (TAIR10)	AT3G11010.1	RLP	lrr
Bna_LepR3	Arabidopsis thaliana (TAIR10)	AT3G11080.1	RLP	lrr
Bna_LepR3	Arabidopsis thaliana (TAIR10)	AT3G28890.1	RLP	lrr
Bna_LepR3	Arabidopsis thaliana (TAIR10)	AT5G25910.1	RLP	lrr
Bna_LepR3	Arabidopsis thaliana (TAIR10)	AT5G27060.1	RLP	lrr
Bna_LepR3	Brassica napus Darmor bzh 4.1.	BnaA09g11190D	NLR	TNL
Bna_LepR3	Brassica napus Darmor bzh 4.1.	BnaC01g31430D	NLR	TNL
Bna_LepR3	Brassica napus Darmor bzh 4.1.	BnaC04g08930D	NLR	CNL
Bna_LepR3	Brassica napus Darmor bzh 4.1.	BnaC09g50760D	NLR	NL
Bna_LepR3	Brassica nigra (vv2)	BniB020414-TA	NLR	TNL
Bna_LepR3	Brassica nigra (vv2)	BniB021030-TA	NLR	TNL
Bna_LepR3	Brassica nigra (vv2)	BniB028796-TA	RLP	lrr
Bna_LepR3	Brassica nigra (vv2)	BniB038823-TA	NLR	TNL
Bna_LepR3	Brassica oleracea TO1000 v2.1.	Bo1g141230.1	RLP	lrr
Bna_LepR3	Brassica oleracea TO1000 v2.1.	Bo1g141270.1	RLP	lrr
Bna_LepR3	Brassica oleracea TO1000 v2.1.	Bo1g141280.1	RLP	lrr
Bna_LepR3	Brassica oleracea TO1000 v2.1.	Bo3g042800.1	RLK	lrr
Bna_LepR3	Brassica oleracea TO1000 v2.1.	Bo3g063960.1	RLP	lrr
Bna_LepR3	Brassica oleracea TO1000 v2.1.	Bo3g074860.1	RLP	lrr
Bna_LepR3	Brassica oleracea TO1000 v2.1.	Bo3g080000.1	RLP	lrr
Bna_LepR3	Brassica oleracea TO1000 v2.1.	Bo3g088600.1	RLP	lrr
Bna_LepR3	Brassica oleracea TO1000 v2.1.	Bo4g158850.1	RLP	lrr
Bna_LepR3	Brassica oleracea TO1000 v2.1.	Bo9g169800.1	RLP	lrr

7

4

4

10

Gene	Org	Closest Genomic Feature	Rga main	Rga subclass
Bna_LepR3	Brassica rapa 3.0	BraA03g026250.3C	RLK	lrr
Bna_LepR3	Brassica rapa 3.0	BraA03g034620.3C	RLP	lrr
Bna_LepR3	Brassica rapa 3.0	BraA03g040130.3C	RLK	lrr
Bna_LepR3	Brassica rapa 3.0	BraA03g042720.3C	RLP	lrr
Bna_LepR3	Brassica rapa 3.0	BraA04g019040.3C	RLP	lrr
Bna_LepR3	Brassica rapa 3.0	BraA04g020320.3C	RLP	lrr
Bna_LepR3	Brassica rapa 3.0	BraA07g033670.3C	RLK	lrr
Bna_LepR3	Brassica rapa 3.0	BraA09g065960.3C	RLP	lrr
Bna_LepR3	Brassica rapa 3.0	BraA10g001010.3C	RLK	other
Bna_LepR3	Brassica rapa 3.0	BraA10g009490.3C	RLK	lrr
Bna_LepR3	Brassica rapa 3.0	BraA10g026360.3C	RLK	other
Bna_LepR3	Brassica rapa 3.0	BraA10g031780.3C	NLR	NL
Bna_LepR3	Camelina sativa (v2)	Csa08g023530.1	RLP	lrr
Bna_LepR3	Camelina sativa (v2)	Csa19g008130.1	RLP	lrr
Bna_LepR3	Camelina sativa (v2)	Csa19g014580.1	RLP	lrr
Bna_LepR3	Camelina sativa (v2)	Csa19g014800.1	RLP	lrr
Bna_LepR3	Camelina sativa (v2)	Csa19g014810.1	RLP	lrr
Bna_LepR3	Camelina sativa (v2)	Csa19g014830.1	RLP	lrr
Bna_LepR3	Camelina sativa (v2)	Csa19g014850.1	RLP	lrr
Bna_LepR3	Camelina sativa (v2)	Csa19g014990.1	RLP	lrr
Bna_LepR3	Camelina sativa (v2)	Csa19g015010.1	RLP	lrr
Bna_LepR3	Camelina sativa (v2)	Csa19g015030.1	RLP	lrr
Bna_LepR3	Camelina sativa (v2)	Csa19g015110.1	RLP	lrr
Bna_LepR3	Camelina sativa (v2)	Csa19g015120.1	RLP	lrr
Bna_LepR3	Camelina sativa (v2)	Csa19g046270.1	RLP	lrr
Bna_LepR3	Raphanus sativus	Rsa1.0_00016.1_g00027.1	RLP	lrr
Bna_LepR3	Raphanus sativus	Rsa1.0_00016.1_g00027.1	RLP	lrr

13

13

2

List of *Rlm2* homolog RGAs per species (>70% similarity)

Gene	Org	Closest Genomic Feature	Rga main	Rga subclass
Bna_Rlm2	Arabidopsis thaliana (TAIR10)	AT2G15042.1	RLP	lrr
Bna_Rlm2	Arabidopsis thaliana (TAIR10)	AT2G15080.1	RLP	lrr
Bna_Rlm2	Arabidopsis thaliana (TAIR10)	AT2G15080.1	RLP	lrr
Bna_Rlm2	Arabidopsis thaliana (TAIR10)	AT3G05660.1	RLP	lrr
Bna_Rlm2	Arabidopsis thaliana (TAIR10)	AT3G11010.1	RLP	lrr
Bna_Rlm2	Arabidopsis thaliana (TAIR10)	AT3G11080.1	RLP	lrr
Bna_Rlm2	Arabidopsis thaliana (TAIR10)	AT3G28890.1	RLP	lrr
Bna_Rlm2	Arabidopsis thaliana (TAIR10)	AT5G25910.1	RLP	lrr
Bna_Rlm2	Arabidopsis thaliana (TAIR10)	AT5G27060.1	RLP	lrr
Bna_Rlm2	Brassica napus Darmor bzh 4.1.	BnaA09g11760D	NLR	TNL
Bna_Rlm2	Brassica napus Darmor bzh 4.1.	BnaC04g08930D	NLR	CNL
Bna_Rlm2	Brassica nigra (vv2)	BniB020414-TA	NLR	TNL
Bna_Rlm2	Brassica nigra (vv2)	BniB021030-TA	NLR	TNL
Bna_Rlm2	Brassica nigra (vv2)	BniB028796-TA	RLP	lrr
Bna_Rlm2	Brassica nigra (vv2)	BniB038823-TA	NLR	TNL
Bna_Rlm2	Brassica oleracea TO1000 v2.1.	Bo1g141230.1	RLP	lrr
Bna_Rlm2	Brassica oleracea TO1000 v2.1.	Bo1g141240.1	RLP	lrr
Bna_Rlm2	Brassica oleracea TO1000 v2.1.	Bo1g141270.1	RLP	lrr
Bna_Rlm2	Brassica oleracea TO1000 v2.1.	Bo1g141280.1	RLP	lrr
Bna_Rlm2	Brassica oleracea TO1000 v2.1.	Bo3g042800.1	RLK	lrr
Bna_Rlm2	Brassica oleracea TO1000 v2.1.	Bo3g063960.1	RLP	lrr
Bna_Rlm2	Brassica oleracea TO1000 v2.1.	Bo3g074860.1	RLP	lrr
Bna_Rlm2	Brassica oleracea TO1000 v2.1.	Bo3g080000.1	RLP	lrr
Bna_Rlm2	Brassica oleracea TO1000 v2.1.	Bo3g088600.1	RLP	lrr
Bna_Rlm2	Brassica oleracea TO1000 v2.1.	Bo4g158850.1	RLP	lrr
Bna_Rlm2	Brassica oleracea TO1000 v2.1.	Bo9g169800.1	RLP	lrr

9

2

4

11

Gene	Org	Closest Genomic Feature	Rga main	Rga subclass
Bna_Rlm2	Brassica rapa 3.0	BraA01g016140.3C	RLK	lrr
Bna_Rlm2	Brassica rapa 3.0	BraA03g009500.3C	RLK	other
Bna_Rlm2	Brassica rapa 3.0	BraA03g026250.3C	RLK	lrr
Bna_Rlm2	Brassica rapa 3.0	BraA03g034620.3C	RLP	lrr
Bna_Rlm2	Brassica rapa 3.0	BraA03g040130.3C	RLK	lrr
Bna_Rlm2	Brassica rapa 3.0	BraA03g042720.3C	RLP	lrr
Bna_Rlm2	Brassica rapa 3.0	BraA04g019040.3C	RLP	lrr
Bna_Rlm2	Brassica rapa 3.0	BraA05g006570.3C	RLK	lrr
Bna_Rlm2	Brassica rapa 3.0	BraA06g038410.3C	RLK	other
Bna_Rlm2	Brassica rapa 3.0	BraA08g002590.3C	RLK	lysm
Bna_Rlm2	Brassica rapa 3.0	BraA10g026360.3C	RLK	other
Bna_Rlm2	Brassica rapa 3.0	BraA10g026360.3C	RLK	other
Bna_Rlm2	Camelina sativa (v2)	Csa08g023530.1	RLP	lrr
Bna_Rlm2	Camelina sativa (v2)	Csa19g008130.1	RLP	lrr
Bna_Rlm2	Camelina sativa (v2)	Csa19g014580.1	RLP	lrr
Bna_Rlm2	Camelina sativa (v2)	Csa19g014800.1	RLP	lrr
Bna_Rlm2	Camelina sativa (v2)	Csa19g014810.1	RLP	lrr
Bna_Rlm2	Camelina sativa (v2)	Csa19g014830.1	RLP	lrr
Bna_Rlm2	Camelina sativa (v2)	Csa19g014850.1	RLP	lrr
Bna_Rlm2	Camelina sativa (v2)	Csa19g014990.1	RLP	lrr
Bna_Rlm2	Camelina sativa (v2)	Csa19g015010.1	RLP	lrr
Bna_Rlm2	Camelina sativa (v2)	Csa19g015030.1	RLP	lrr
Bna_Rlm2	Camelina sativa (v2)	Csa19g015110.1	RLP	lrr
Bna_Rlm2	Camelina sativa (v2)	Csa19g015120.1	RLP	lrr
Bna_Rlm2	Camelina sativa (v2)	Csa19g046270.1	RLP	lrr
Bna_Rlm2	Raphanus sativus	Rsa1.0_00013.1_g00043.1	RLP	lrr
Bna_Rlm2	Raphanus sativus	Rsa1.0_00016.1_g00027.1	RLP	lrr
Bna_Rlm2	Raphanus sativus	Rsa1.0_00179.1_g00063.1	RLP	lrr

12

13

3