

# Major gene resistance: Molecular Marker Update

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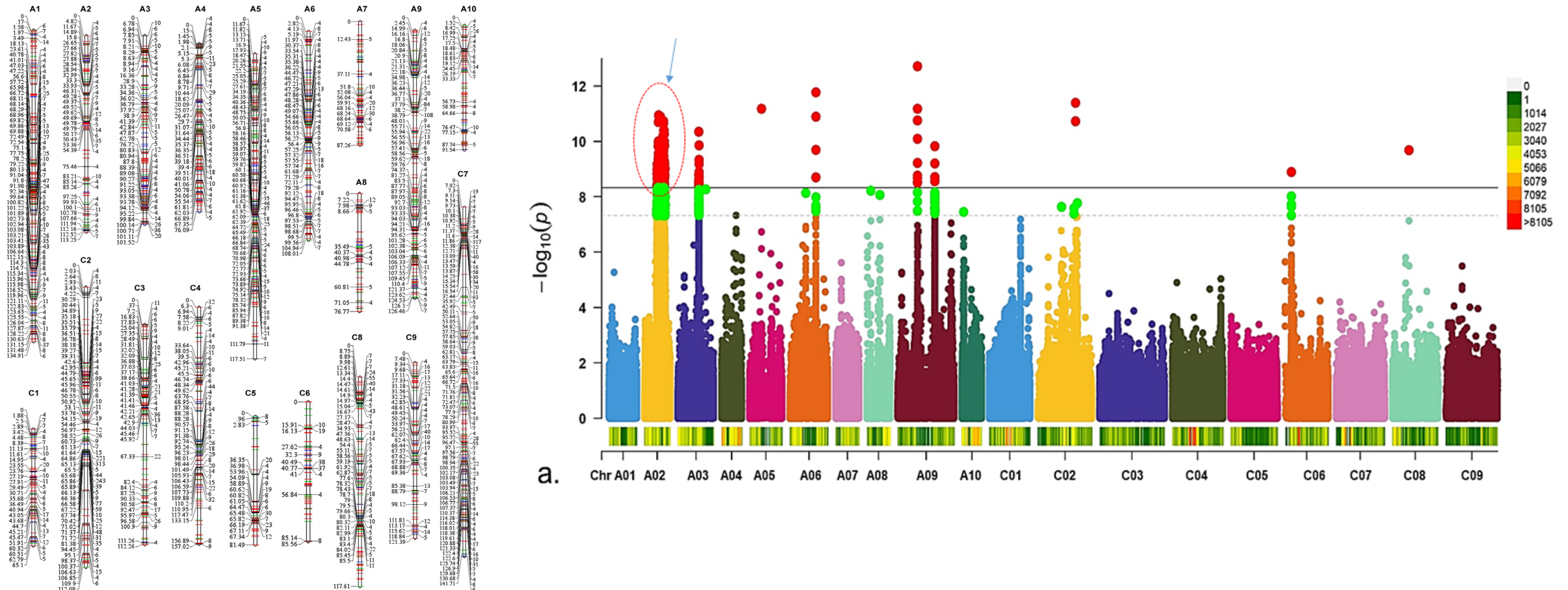
- Identify candidate genes for genetically mapped resistance genes
- Develop molecular markers for routine screening of blackleg resistance genes
- Deliver novel sources of qualitative blackleg resistance

# Resistance genes

- Chromosome A02: *LepR1*
- Chromosome A07: *Rlm1*, *Rlm3*, *Rlm4*, *Rlm7*, *Rlm9*
- Chromosome A10: *LepR2*, *Rlm2*, *LepR3*
- Chromosome C03: *Rlm13*, *Rlm6* - *napus*

# Gene mapping using high-density SNP markers

- 60 and 90K SNPs and WGRS SNPs
- GWAS and QTL mapping
- Introgression detection
- Used for species confirmation



# Resistance (*R*) genes

## TM-LRR (Transmembrane leucine-rich-repeats)



### RLP

(Receptor-like proteins)

### RLK

(Receptor-like kinase)



## NLR (Nucleotide binding site-leucine-rich repeats)



### CN

(Coil coiled nucleotide-binding site)

### CNL

(Coil Coiled nucleotide-binding site-leucine rich repeats)

### NBS

(Nucleotide-binding site)

### NL

(Nucleotide binding site Leucine-rich repeats)

### TN

(Toll/Interleukin-1 receptor-NBS)

### TNL

(Toll/Interleukin-1 receptor-NBS-LRR)

### TX

(TIR-unknown domain)

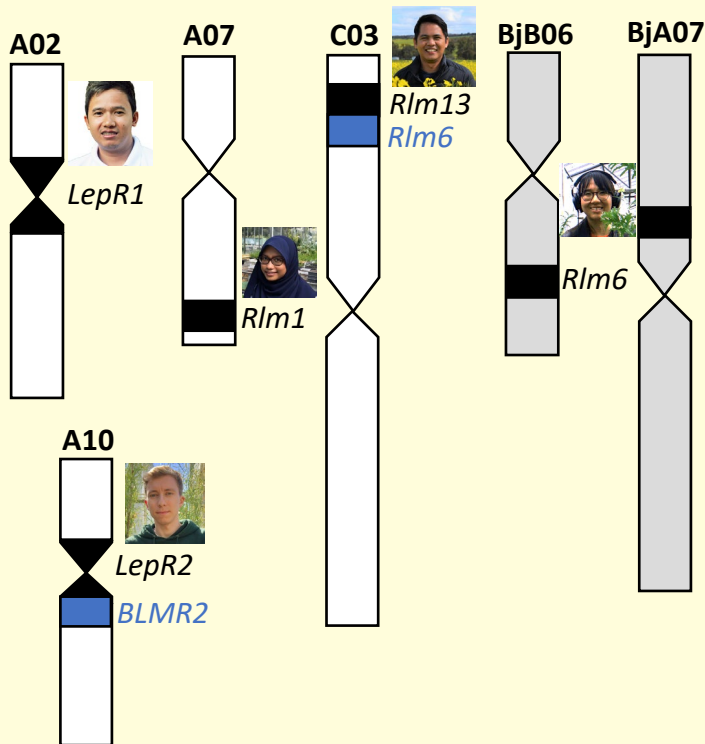
### OTHER

(TIR-unknown domain)

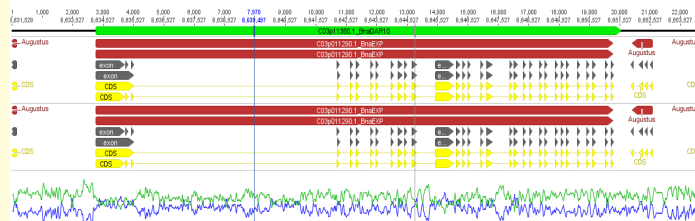
# On-going validation of candidate blackleg *R*-genes

## Genetic mapping

QTL mapping and GWAS using the 60 and 90K and WGRS SNPs



## *R*-gene mining in reference genomes



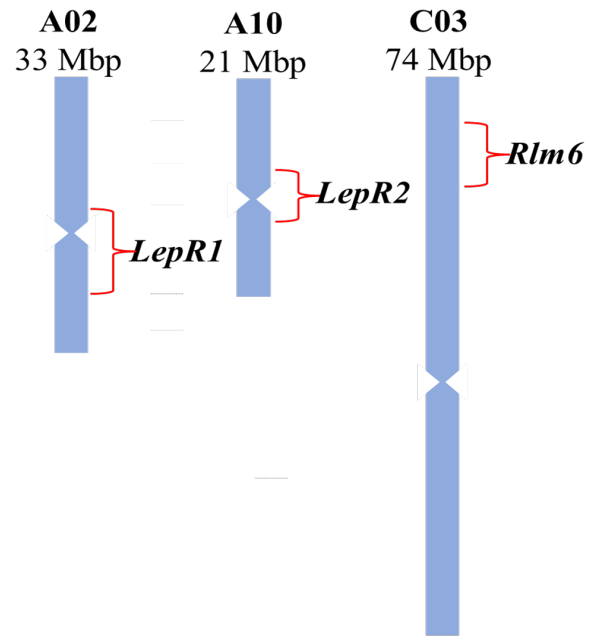
Locus	Candidate genes
<i>Rlm1</i>	29
<i>Rlm6-napus</i>	44
<i>Rlm13</i>	28
<i>LepR1</i>	30
<i>LepR2</i>	19
<i>BLMR2</i>	2
<i>Rlm6-junceae</i>	16

## Gene validation

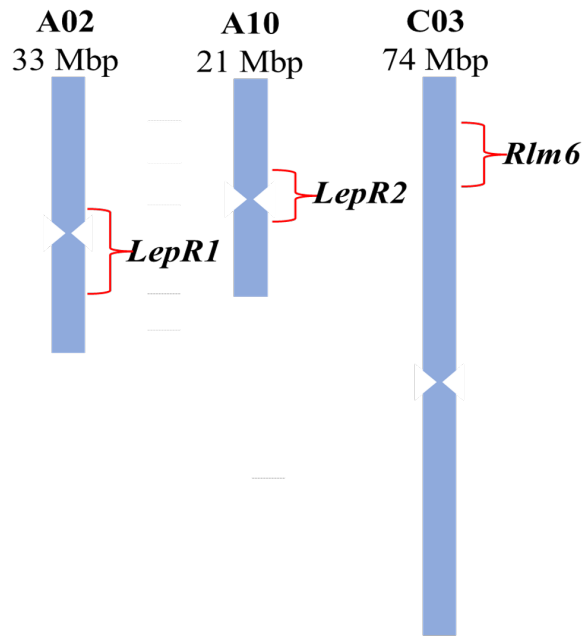
- PCR amplification
- nCATs
- WGRS
- Sequencing (Nanopore, MiSeq and Sanger)

Functional validation

# Current targets



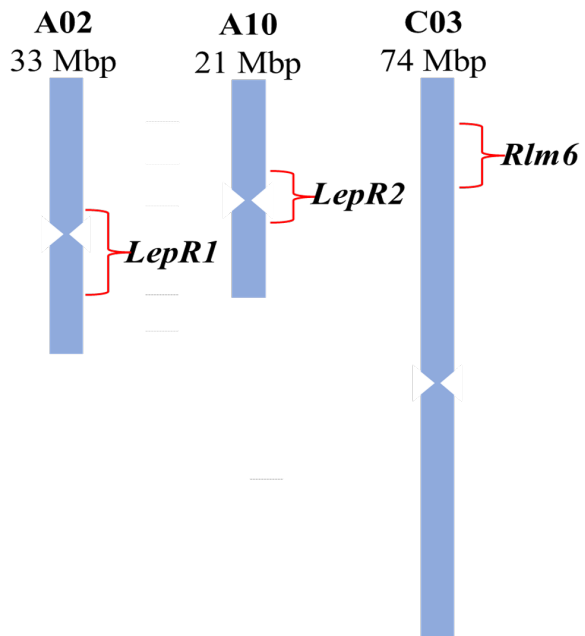
# Current targets



Major gene	Total number of candidate <i>R</i> genes	Strong candidate genes
<i>LepR1</i>	30	1
<i>LepR2</i>	19	5
<i>Rlm6</i>	60	1

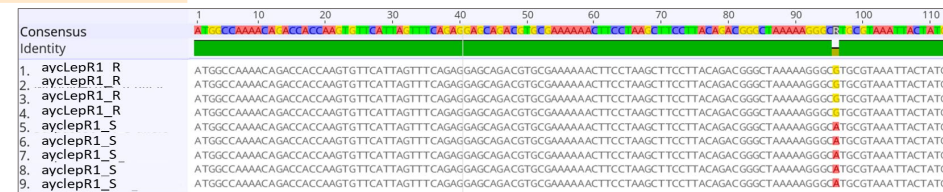


# Current targets

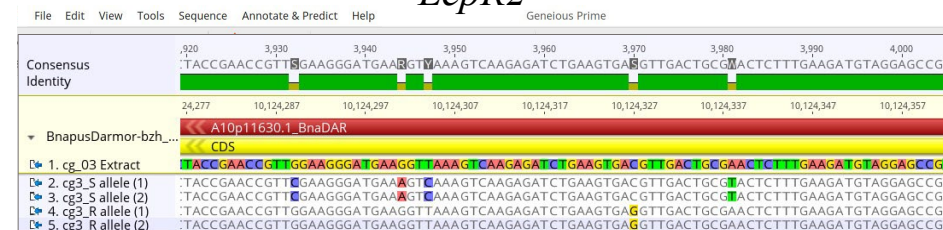


Major gene	Total number of candidate <i>R</i> genes	Strong candidate genes
<i>LepR1</i>	30	1
<i>LepR2</i>	19	5
<i>Rlm6</i>	60	1

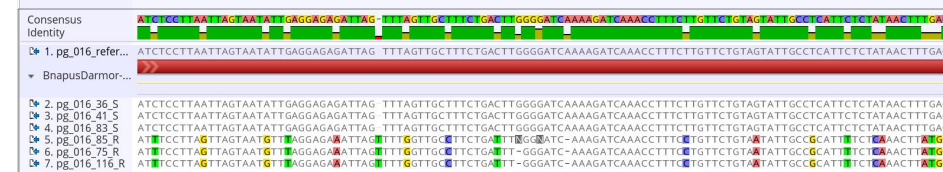
*LepR1*



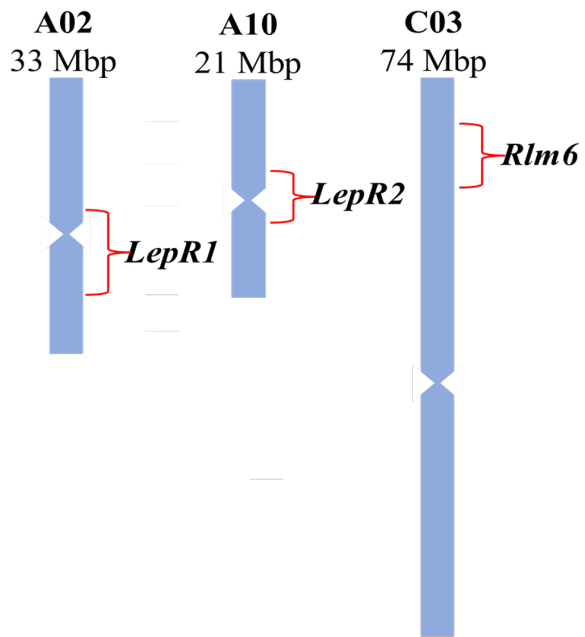
*LepR2*



*Rlm6*



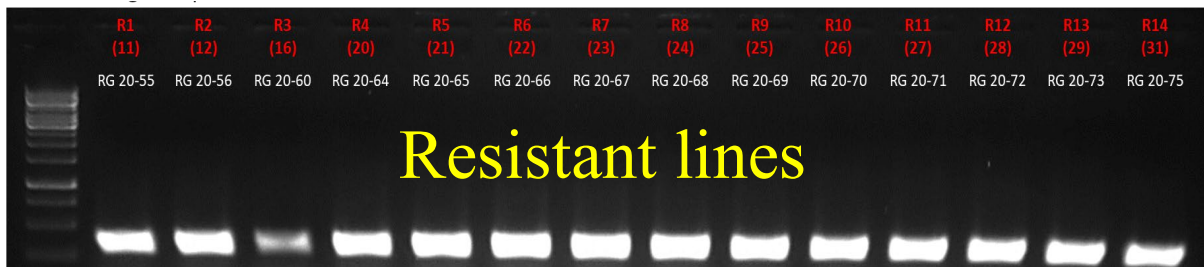
# Current targets



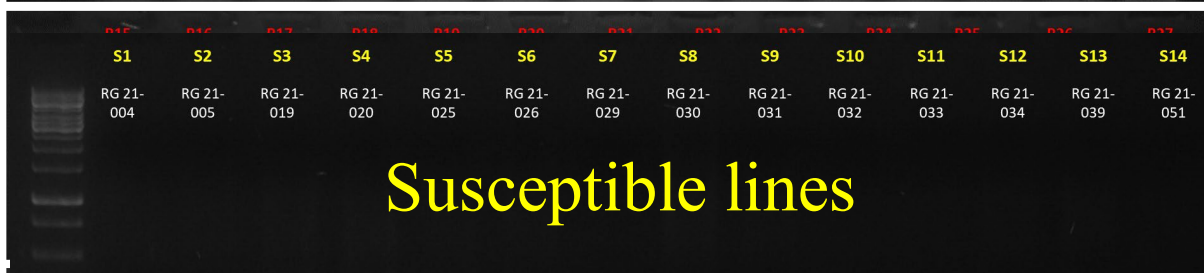
Major gene	Total number of candidate <i>R</i> genes	Strong candidate genes
<i>LepR1</i>	30	1
<i>LepR2</i>	19	5
<i>Rlm6</i>	60	1

*LepR1*

Consensus Identity	1	10	20	30	40	50	60	70	80	90	100	110
1. <i>aycLepR1_R</i>	ATGGCCAAAACAGACCACCAAGTGTTCATTAGTTTCAGAGGAGCAGACGTGCGAAAAA	ACTTCTTAAGCTTCTTACAGACGGCTAAAAAGGGCGTGGTAAATTA	CTATGT									
2. <i>aycLepR1_R</i>	ATGGCCAAAACAGACCACCAAGTGTTCATTAGTTTCAGAGGAGCAGACGTGCGAAAAA	ACTTCTTAAGCTTCTTACAGACGGCTAAAAAGGGCGTGGTAAATTA	CTATGT									
3. <i>aycLepR1_R</i>	ATGGCCAAAACAGACCACCAAGTGTTCATTAGTTTCAGAGGAGCAGACGTGCGAAAAA	ACTTCTTAAGCTTCTTACAGACGGCTAAAAAGGGCGTGGTAAATTA	CTATGT									
4. <i>aycLepR1_S</i>	ATGGCCAAAACAGACCACCAAGTGTTCATTAGTTTCAGAGGAGCAGACGTGCGAAAAA	ACTTCTTAAGCTTCTTACAGACGGCTAAAAAGGGCGTGGTAAATTA	CTATGT									
5. <i>aycLepR1_S</i>	ATGGCCAAAACAGACCACCAAGTGTTCATTAGTTTCAGAGGAGCAGACGTGCGAAAAA	ACTTCTTAAGCTTCTTACAGACGGCTAAAAAGGGCGTGGTAAATTA	CTATGT									
6. <i>aycLepR1_S</i>	ATGGCCAAAACAGACCACCAAGTGTTCATTAGTTTCAGAGGAGCAGACGTGCGAAAAA	ACTTCTTAAGCTTCTTACAGACGGCTAAAAAGGGCGTGGTAAATTA	CTATGT									
7. <i>aycLepR1_S</i>	ATGGCCAAAACAGACCACCAAGTGTTCATTAGTTTCAGAGGAGCAGACGTGCGAAAAA	ACTTCTTAAGCTTCTTACAGACGGCTAAAAAGGGCGTGGTAAATTA	CTATGT									
8. <i>aycLepR1_S</i>	ATGGCCAAAACAGACCACCAAGTGTTCATTAGTTTCAGAGGAGCAGACGTGCGAAAAA	ACTTCTTAAGCTTCTTACAGACGGCTAAAAAGGGCGTGGTAAATTA	CTATGT									
9. <i>aycLepR1_S</i>	ATGGCCAAAACAGACCACCAAGTGTTCATTAGTTTCAGAGGAGCAGACGTGCGAAAAA	ACTTCTTAAGCTTCTTACAGACGGCTAAAAAGGGCGTGGTAAATTA	CTATGT									



Resistant lines



Susceptible lines

*LepR2*

File Edit View Tools Sequence Annotate & Predict Help Genescope Prime

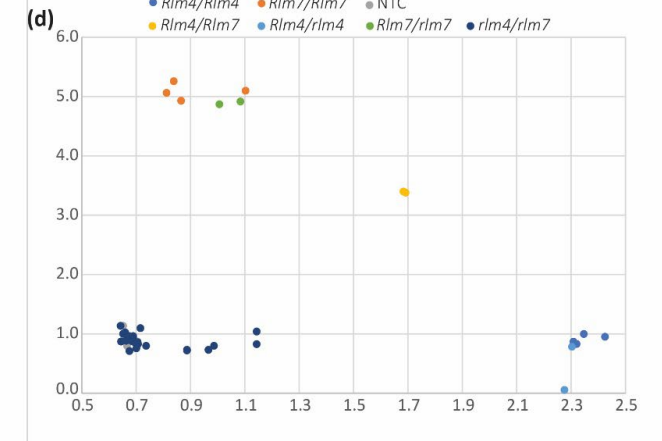
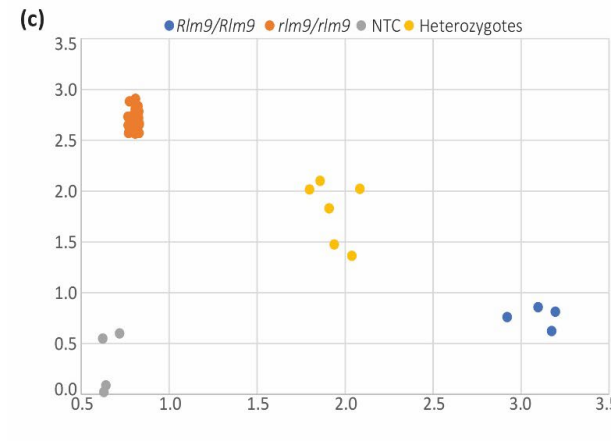
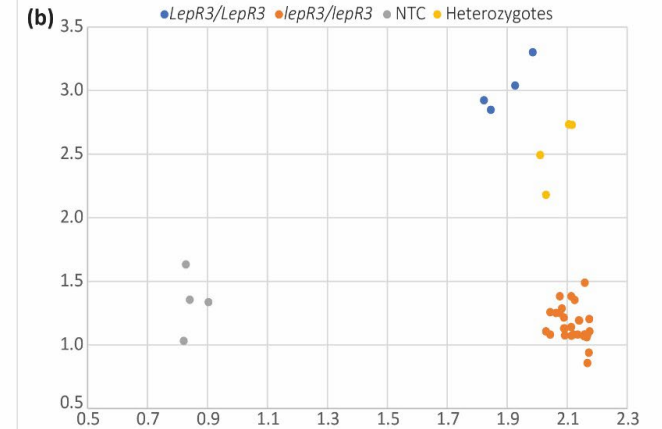
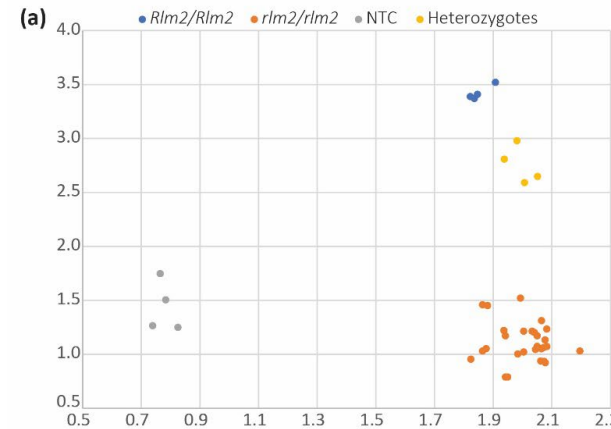
Consensus Identity	,920	3,930	3,940	3,950	3,960	3,970	3,980	3,990	4,000
	TACCGAACCGTTTGAAGGGATGAAGGTAAAAGTCAAGAGATCTGAAGTGAAGTTGACTGCGAACTCTTTGAAGATGTAGGAGCCGT								
24,277	10,124,287	10,124,297	10,124,307	10,124,317	10,124,327	10,124,337	10,124,347	10,124,357	
BnopusDarmor-bzh... A10p11630.1_BnaDAR CDS 1. <i>cg_03</i> Extract 2. <i>cg3_S</i> allele (1) 3. <i>cg3_S</i> allele (2) 4. <i>cg3_R</i> allele (1) 5. <i>cg3_R</i> allele (2)									

*Rlm6*

Consensus Identity	140	150	160	170	180	190	200	210	220	230	240	250
	ATCTCCCTAATTAGTAATATTGAGGAGAGATTAGTTTAGTGTCTTCTGACTTGGGGATCAAAGATCAAACCTTTCTGTCTGTAGTATTGCCTCATTCTATAACTTTGACT											
1. <i>pg_016_refer...</i>	ATCTCCCTAATTAGTAATATTGAGGAGAGATTAGTTTAGTGTCTTCTGACTTGGGGATCAAAGATCAAACCTTTCTGTCTGTAGTATTGCCTCATTCTATAACTTTGACT											
BnopusDarmor... 2. <i>pg_016_36_S</i> 3. <i>pg_016_41_S</i> 4. <i>pg_016_83_S</i> 5. <i>pg_016_85_R</i> 6. <i>pg_016_75_R</i> 7. <i>pg_016_116_R</i>												

# KASP Markers

- Developed: *LepR3*, *Rlm2*, *Rlm3*, *Rlm4*, *Rlm7*, *Rlm9*
- Under development: *LepR1*, *Rlm6-napus*



# Novel Resistance

- SN lines
  - SN1, 2, 5, 18, 19 (also QR)
- YM x westar
  - YM 6, 7, 14

# Conclusion

- We have identified strong candidate genes for *LepR1*, *LepR2* and *Rlm6*, which are currently undergoing further validation.
- Screening markers can be used to rapidly identify the genotypes of *B. napus* lines.
- Conduct functional tests of the strong candidate genes using transgenic or genome editing approaches
- Develop kompetitive allele-specific PCR (KASP) markers for use in marker-assisted selection.