

White blister rust of mustards

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Developing a genetics and genomics strategy for sustainable resistance to white rust (WR) in *Brassica juncea*

AIMS

- 1. Develop genomic resources for the identification of Brassica and Arabidopsis genes that control WR resistance**
 1. Identify candidate genes for WR resistance in *B. juncea*
 2. Map new resistances to WR in diploid brassicas
- 2. Develop pathogen-based information**
 1. Study the diversity of *A. candida* in Indian populations and compare with other countries
 2. Study other major brassica diseases including downy mildew (*Hyaloperonospora brassicae*)

In India: Delhi-U; Amity-U; GBPUAT-U

In the UK: Warwick U; TSL-Norwich, Bath U

WR disease symptoms



Oomycete, obligate parasite.

Local and systemic infections, malformations (hyperplasia and hypertrophy)

Frequently found in association with downy mildew



White rust in mustard (*Brassica juncea*) Indo-UK partnership

In India

- Rape-seed mustard is important oilseed crop.
- 5.5 Mha and 6.4MT in India (28% of world acreage and 20% world production)
- Resistance loci mapped in two European lines
- Loci are non-allelic and confer different level of resistance to Ac2v
 - Heera partial resistance
 - Donskaja-IV full resistance

In the UK

- Mustard crops:
 - brown mustard (300ha)
 - white mustard (400ha)
- Areas around Peterborough
- *B. juncea* grown for green manure
- Work in progress: identification and characterisation of sources of resistance



Pathotypes of *A. candida* in India

	Meerut	Morena	Pant.	IARI	Kanpur
		Bangalore		Katiti	Bihar
				B. nigra	Karnataka
				B. napus	Hisar
					Simour
					Alwar
Cutlass	3.6	3.6	0	0	0
Donskaja	0	0	3.7	0	0
Heera	3.6	0	3.6	3.6	0
Varuna	3.7	3.7	3.7	3.7	3.7
RH-30	3.7	3.7	3.7	3.7	3.6

Screening of *Brassicaceae* with three WR isolates

Selection of accessions from three Gene banks:

- USDA, USA (30 PI accessions)
- CGN, The Netherlands (32 accessions)
- GRU, Wellesbourne, Warwick (61 accessions)
- India – Heera and Donskaja IV
- Commercial lines

Total of >150 accessions

Flow cytometry results for 50 accessions

Origin	Accession Name/Number (this study)	Received as	DNA ratio	Conclusion
Controls	Burgonde, FBLM2, Cutlass	<i>B. juncea</i>	1.23	<i>B. juncea</i> control
D. Pental selection	120 (Donskaja-IV)	<i>B. juncea</i>	1.22	<i>B. juncea</i>
Field	Sutton	<i>B. juncea</i>	1.22	<i>B. juncea</i>
PI	8	<i>B. juncea</i>	1.42	<i>B. carinata</i>
PI	10, 16	<i>B. juncea</i>	1.23-1.25	<i>B. juncea</i>
PI	29	<i>B. juncea</i>	0.62	<i>B. rapa</i>
CGN	31	<i>B. juncea</i>	1.42	<i>B. carinata</i>
CGN	43, 45, 46, 48, 59, 60, 62	<i>B. juncea</i>	1.20-1.22	<i>B. juncea</i>
CGN	44	<i>B. juncea</i>	0.60	<i>B. rapa</i>
Warwick GRU	67, 69, 73, 75, 90, 95, 99, 101, 125	<i>B. juncea</i>	1.20-1.25	<i>B. juncea</i>
Warwick GRU	63, 65, 66, 96, 97, 107, 108, 110, 112, 113, 114, 117	<i>B. juncea</i>	0.59-0.67	<i>B. rapa</i>
Warwick GRU	100	<i>B. juncea</i>	1.34	<i>B. napus</i>
Commercial line	131, 135, 144	Various mustards	1.22-1.25	<i>B. juncea</i>
Commercial line	132, 133, 136, 145	Various mustards	0.59-0.60	<i>B. rapa</i>

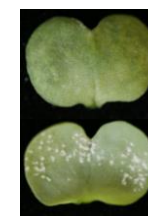
Results of WR screening

	Lines tested	AcBjDC	AcBj12	AcSa9
<i>S. alba</i>	12 lines	I/R	I/S	I/S
<i>B. oleracea</i>	Lateman	R	I	I/S
	19 lines	R	R	R
<i>B. rapa</i>	Torch	I/R	I	I/S
	JRT	R	I	R/I
	10 accessions	I/R	S/I/R	I/R
	5 accessions	R	R	R
<i>B. carinata</i>	3 lines	R	R	R
<i>B. napus</i>	3 lines	R	R	R
<i>A. thaliana</i>	Col5	R	R	R
	Magic23, 329	S	S	S
<i>Capsella b-p</i>	2 accessions	R	R	R
<i>R. sativus</i>	4 lines	R	R	R

AcBjDC



AcBj12



AcSa9



B. juncea
Sutton



B. carinata
PIC1



Selected *B. juncea* lines with differential reactions

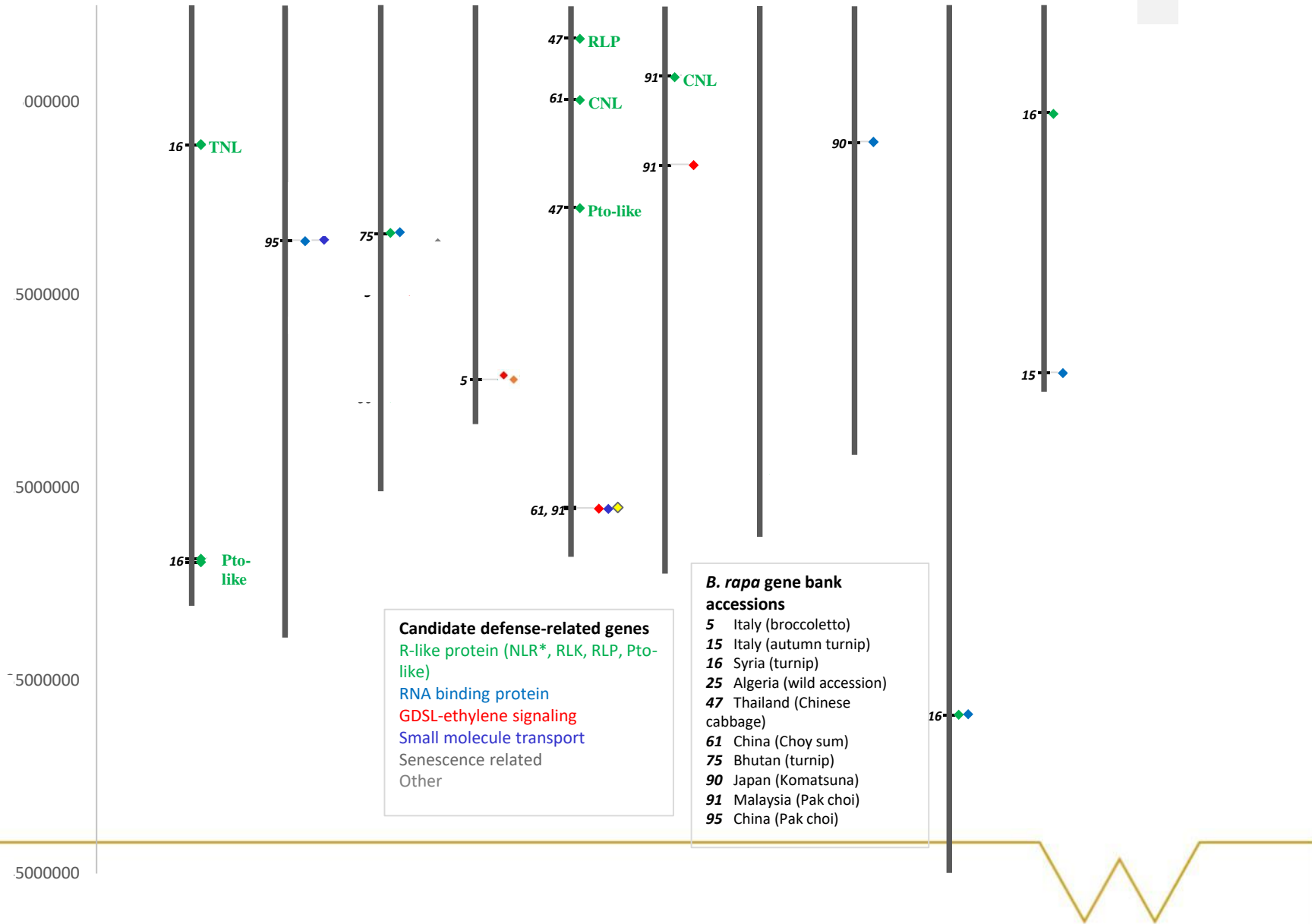
			Interactions with <i>Albugo candida</i> isolate		
			AcSa9	AcBj12	AcBjDC
Line number in this study	Seed colour	Predicted R-genes	Predicted effector genes		
			E1 or (E1+E2+E3) or (E1+E2+E3+E4)	E3 or (E3+E4)	E2 or (E2+E4)
1A	Yellow	None	+	+	+
126	Brown				
127, 176	Brown	R1	-	+	+
43	Yellow	(R1+R2) or	-	+	-
167, 179, 181	Brown	R2			
156	Yellow	(R1+R3) or R3	-	-	+
173	Brown	(R1+R2+R3) or (R2+R3) or R4	-	-	-

Selected plants were self-pollinated to obtain uniform lines;
Crosses are being done; F₁s to be tested and self-pollinated to obtain F₂s

Using Genotyping-by-sequencing (GBS) to map WR resistance

- *B. rapa* accessions from the Warwick GRU
- Screened with AcBj12 and AcBjDC
- Individual R-plants and bulk susceptible plants
- Identified SNPs for dominant resistance alleles
- Evidence based on
 - single allele (homozygous for one bp) in susceptible bulks
 - heterozygous or homozygous for the alternative bp in resistant individuals
- Candidate defense-related genes located within 100kb (50kb either side of the SNP).

Priority GBS_SNPs and nearest candidate genes (using AcBj12 and Phytozome reference genome)



Sequencing of WR isolates from England

- ITS and specific markers sequencing – showed that isolates are *A. candida*
- *Note: A. candida* Ac2V from *B. juncea*, Canada, has been sequenced (including PacBio) - Good to perform reference guided assembly
- *A. candida* from brown mustard (*B. juncea*), UK
 - AcBjDC: MiSeq and Minlon @Warwick
 - AcBj12: HiSeq @Earlham: 12k contigs; largest contig 252 kb
Minlon @Warwick
- *A. candida* from white mustard (*Sinapis alba*), UK
 - AcSa9: HiSeq @Earlham: 35k contigs; largest contig 295 kb
- Extra Sequencing: *Albugo* from oil radish: MiSeq @Warwick

Sequencing of *Hyaloperonospora brassicae* from England

Hpb717 downy mildew from *B. oleracea*

Pathotype 3, one of the most common in our collection

MiSeq at Warwick Life Sciences: 14424743 raw reads

Data analysis: 14418139 trimmed reads remained

- The best assembly: Hpb (kmer=147) 174141 contigs,
- N50 1920, a longest contig of 131kb
- Estimated size of 163Mb

PacBio sequencing assembled at Earlham

Add RNASeq results

HpbBj downy mildew from *B. juncea*

HiSeq at Earlham

Best assembly: 59000 contigs, N50 1880, longest contig of 485kb

Estimated size of 139Mb

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WARWICK
THE UNIVERSITY OF WARWICK

