Short communication

An avirulence gene, *AvrLmJ1*, from the blackleg fungus, *Leptosphaeria maculans*, confers avirulence to *Brassica juncea* cultivars

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SUMMARY

The fungus *Leptosphaeria maculans* causes blackleg of *Brassica* species. Here, we report the mapping and subsequent cloning of an avirulence gene from *L. maculans*. This gene, termed *AvrLmJ1*, confers avirulence towards all three *Brassica juncea* cultivars tested. Analysis of RNA-seq data showed that *AvrLmJ1* is housed in a region of the *L. maculans* genome which contains only one gene that is highly expressed *in planta*. The closest genes are 57 and 33 kb away and, like other avirulence genes of *L. maculans*, *AvrLmJ1* is located within an AT-rich, gene-poor region of the genome. The encoded protein is 141 amino acids, has a predicted signal peptide and is cysteine rich. Two virulent isolates contain a premature stop codon in *AvrLmJ1*. Complementation of an isolate that forms cotyledonary lesions on *B. juncea* with the wild-type allele of *AvrLmJ1* confers avirulence towards all three *B. juncea* cultivars tested, suggesting that the gene may confer species-specific avirulence activity.

The dothideomycete *Leptosphaeria maculans* causes significant yield losses in *Brassica* species, particularly *B. napus* (canola or oilseed rape), worldwide (Fitt *et al.*, 2006). *Brassica* species comprise important oilseed and vegetable crops. They are derived from three ancestral diploid species, whose genomes are AA, BB and CC; these have been intercrossed to generate amphidiploid species. The genomic relationships between these species are described as the 'Triangle of U' (U, 1935). Species with the B genome are generally more resistant than *B. napus* (AACC genome) to *L. maculans* (Plieske *et al.*, 1998).

Field populations of *L. maculans* rapidly adapt to selection pressure from the sowing of cultivars of *B. napus* with major gene resistance, and can overcome resistance within a few years of release of a cultivar (Rouxel *et al.*, 2003; Sprague *et al.*, 2006). This pathogen shows gene-for-gene interactions with *Brassica* hosts.

So far, only one resistance gene, *LepR3*, has been cloned (Larkan *et al.*, 2013). Four cultivar-specific avirulence genes, *AvrLm1*, *AvrLm4-7*, *AvrLm6* and *AvrLm11*, which all encode small secreted proteins, have been cloned (Balesdent *et al.*, 2013; Fudal *et al.*, 2007; Gout *et al.*, 2006; Parlangue *et al.*, 2009). *AvrLm1* confers avirulence towards *B. napus* resistance gene *Rlm1*, whereas *AvrLm6* confers avirulence towards resistance gene *Rlm6*. *AvrLm4-7* confers avirulence towards two *B. napus* resistance genes, *Rlm4* and *Rlm7* (Parlangue *et al.*, 2009). The most recently cloned avirulence gene, *AvrLm11*, is located on a dispensable chromosome and confers avirulence towards resistance gene *Rlm11* of *B. rapa* (AA genome) (Balesdent *et al.*, 2013).

Avirulence genes *AvrLm1*, *AvrLm6*, *AvrLm4-7* and *AvrLm11*, as well as other effectors, are located in gene-poor regions of the *L. maculans* genome, which is compartmentalized into discrete alternating blocks of DNA (isochores) which are either gene rich or gene poor (Rouxel *et al.*, 2011). The gene-poor regions are AT rich and comprise repetitive DNA derived from transposable elements which have been inactivated by a mutation process, repeat-induced point (RIP) mutation, which confers C to T transitions in multicopy loci or repetitive DNA premeiotically (Cambareri *et al.*, 1989). This location provides an unstable genomic environment, enabling avirulence and other effector genes to be lost, gained or RIP mutated much more readily than genes elsewhere in the genome.

As well as cultivar-specific avirulence genes, a putative species-specific avirulence locus has been reported for *L. maculans*. Chen *et al.* (1996) showed that the ability of an *L. maculans* isolate (IBCN18) to form cotyledonary lesions on *B. juncea* (AABB genome) segregated as a single locus. Furthermore, this isolate, as well as several others, attacked all (92) *B. juncea* lines tested (Chen *et al.*, 1996; Purwantara *et al.*, 1998). We report here the cloning of this gene in *L. maculans*.

Initially, we confirmed the *juncea*-attacking phenotype of two isolates [IBCN18 (previously named M1) and V4] described by Purwantara *et al.* (1998). As expected, these two isolates formed cotyledonary lesions on three *B. juncea* cultivars tested (Aurea, Stoke and Forge), and on Westar, a universally susceptible

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Table 1 Pathogenicity scores for seedlings of *Brassica* cultivars after inoculation with *Leptosphaeria maculans* isolates.

Isolate	Avirulence genotype*	Average pathogenicity score (standard error)†			
		<i>B. juncea</i> cv. Aurea	<i>B. juncea</i> cv. Stoke	<i>B. juncea</i> cv. Forge	<i>B. napus</i> cv. Westar
IBCN18	<i>AvrLm1</i> , 2, 4, 7, <i>LepR3</i>	5.4 (1.7)	5.8 (1.5)	7.0 (0.0)	6.8 (0.5)
V4	<i>AvrLm1</i> , 4, 7, <i>LepR3</i> (2, 3, 8, 9)	5.5 (1.2)	5.1 (1.7)	N/T	7.1 (0.6)
IBCN13	<i>AvrLm2</i> , 5, 6, 9, <i>LepR3</i>	1.7 (0.2)	1.9 (0.3)	1.0 (0.0)	5.8 (1.6)
IBCN15	<i>AvrLm5</i> , 6, 8, <i>LepR3</i>	2.2 (0.9)	1.9 (0.7)	1.0 (0.0)	7.0 (0.0)
IBCN16	<i>AvrLm5</i> , 6, 8, <i>LepR3</i>	1.1 (0.4)	2.2 (1.2)	3.7 (1.3)	7.0 (0.0)
IBCN17	<i>AvrLm4</i> , 5, 6, 7, 8, <i>LepR3</i>	1.0 (0.0)	1.2 (0.2)	1.0 (0.0)	6.8 (0.6)
IBCN75	<i>AvrLm1</i> , 5, 6, 8, <i>LepR3</i>	1.7 (0.3)	1.8 (0.4)	1.0 (0.0)	7.5 (0.7)
IBCN76	<i>AvrLm1</i> , 3, 5, 6, 8, <i>LepR3</i>	1.1 (0.3)	1.7 (0.5)	1.0 (0.0)	7.2 (0.3)
PHW1223	<i>AvrLm5</i> , 6, 8, 9, <i>LepR3</i>	2.1 (0.6)	1.6 (0.6)	1.2 (0.3)	6.2 (1.1)
D8	<i>AvrLm5</i> , 7 (8)	2.7 (1.2)	1.3 (0.3)	1.7 (0.5)	7.1 (0.9)
D9	<i>AvrLm5</i> , 6, 7 (8)	1.1 (0.6)	1.1 (0.2)	1.0 (0.0)	7.9 (0.7)
D13	<i>AvrLm4</i> , 6, 7 (5, 8)	2.1 (1.2)	1.9 (0.5)	1.0 (0.0)	6.8 (0.2)
D14	<i>AvrLm1</i> , 7, <i>LepR3</i> (5, 8)	2.2 (1.5)	2.4 (1.1)	2.6 (1.1)	7.2 (0.3)
LM691	<i>AvrLm1</i> , 6 (2, 5, 7, 8, 9, <i>LepR3</i>)	1.0 (0.0)	1.0 (0.0)	N/T	5.9 (1.5)
LM535	<i>AvrLm1</i> , 6 (2, 5, 7, 8, 9, <i>LepR3</i>)	1.7 (0.3)	1.4 (0.3)	N/T	5.9 (1.2)
06P041	<i>AvrLm5</i> (8, 9, <i>LepR3</i>)	1.8 (0.4)	1.8 (0.6)	N/T	7.1 (0.3)
04P013	<i>AvrLm1</i> , 5, 7, <i>LepR3</i> (2, 8)	1.6 (0.2)	1.8 (0.5)	N/T	6.2 (1.1)

*The avirulence genotype indicates the loci for which isolates have previously been characterized as avirulent; loci listed in parentheses are those for which the allele has not been characterized.

†Conidia (10^4) of *L. maculans* were inoculated onto cotyledons (four inoculations per cotyledon) of each cultivar (eight plants per isolate–cultivar combination) and symptoms were assessed at 14 days post-inoculation (dpi) on a scale of '0' (no darkening around wounds) to '9' (large grey–green lesions with prolific sporulation) (Koch *et al.*, 1991). Average pathogenicity scores > 5.0 indicate a virulent phenotype, whereas scores < 5.0 indicate an avirulent phenotype.

N/T, not tested.

B. napus cultivar (Table 1). An additional 15 isolates were all avirulent on the three *B. juncea* cultivars, and virulent on cv. Westar. Two fungal crosses were established to map (a)virulence towards *B. juncea*. Progeny (58) from a cross between the *juncea*-attacking isolate, IBCN18, and the nonattacking isolate, IBCN17, have been generated previously and screened for virulence towards *B. juncea* cv. Stoke. A single gene for avirulence segregated in this cross (Cozijnsen *et al.*, 2000). A second cross was generated between IBCN18 and the nonattacking isolate, LM691. The resulting 69 progeny from the second cross were screened for avirulence towards *B. juncea* cultivars Aurea and Stoke. Thirty-seven of the progeny were avirulent and 32 were virulent on both cultivars. This 1:1 ratio supported the hypothesis of a single avirulence gene segregating for virulence towards the *B. juncea* cultivars ($\chi^2 = 0.36$, $0.7 > P > 0.5$). Fifty-three mini- and microsatellite markers developed from across the entire genome sequence of *L. maculans* isolate v23.1.3 (Rouxel *et al.*, 2011) were screened against the progeny of this cross, and a linkage map was developed using MapManager QTX Software (Manly, 1993). Molecular markers for avirulence genes, *AvrLm1*, *AvrLm6* and *AvrLm4-7*, and the mating-type (*MAT*) locus, were included. Six markers, one of which was *AvrLm6*, from Super Contig 6 formed a linkage group [logarithm of the odds (LOD) scores > 3.0] with the avirulence phenotype on *juncea* (Fig. 1). Deletion of the *AvrLm6* gene confers virulence towards resistance gene *Rlm6* (Fudal *et al.*, 2009; Van de Wouw *et al.*, 2010). This resistance gene is reported to have been introgressed into *B. napus* from *B. juncea* (Chevre

Fig. 1 Mapping the avirulence gene, *AvrLm6*, of *Leptosphaeria maculans*. Sixty-nine progeny in a cross between the *Brassica juncea* attacking isolate, IBCN18, and nonattacking isolate, LM691, were generated and screened for avirulence [*Avr(Juncea)*] towards *B. juncea* cultivars Aurea and Stoke, and also with molecular markers derived from the reference genome. The *AvrLm6* gene is linked [logarithm of the odds (LOD) score > 3.0] to markers on Super Contig 6 of the genome. The distance (cM) for each marker from the *Avr(Juncea)* locus is shown.

Table 2 Oligonucleotide primers.

Name	Sequence (5' to 3')	Fragment size (bp)	Purpose
AvrLm6F	TCAATTGTCTGTCAAGTTATGGA	677	Molecular marker
AvrLm6R	CCAGTTTGAACCGTAGAGGTAGCA		
AvrLm6CloningF	TATAGGATCCAATCGGCAGCTTGATACGAG	2721	Cloning AvrLm6
AvrLm6CloningR	GCGCTCTAGATTATCGACGAACGGAGCAC		
LemaT070880F	ACAACCACTCTTCTCACAGT	479	Sequencing and RFLP marker
LemaT070880R	TGGTTTGGGTAAAGTTGTCCT		
Lema_uP070880 CloningF	GGCGAGGCCTTTTAAAGGGGTAGTGCCTTGTT	1819	Cloning Lema_uP070880.2
Lema_uP070880 CloningR	GGCGGGATCCAAAGTACCCTACCCCTACCTAAGC		

RFLP, restriction fragment length polymorphism.

et al., 1997), making *AvrLm6* a candidate as a gene conferring avirulence towards *B. juncea* cultivars.

A complementation construct was generated and used to test whether *AvrLm6* confers avirulence towards *B. juncea* cultivars Aurea, Stoke and Forge. A DNA fragment (2785 bp) containing *AvrLm6* (580 bp) with up- and downstream regions (955 and 1250 bp, respectively) was PCR amplified from genomic DNA of the sequenced isolate, v23.1.3, using primers with restriction sites on the ends (Table 2). This fragment was cloned into plasmid pZP-Nat, which contains the nourseothricin acetyltransferase gene, using *Bam*HI and *Xba*I (Elliott and Howlett, 2006). The resulting plasmid (11 271 bp) was transformed into isolate IBCN18 using *Agrobacterium*-mediated transformation (Gardiner and Howlett, 2004). All four nourseothricin-resistant transformants tested had become avirulent on *B. juncea* cultivars Aurea, Stoke and Forge, but were still virulent on cv. Westar (Fig. 2). Although these data confirm that *AvrLm6* is involved in avirulence towards *B. juncea*, it is unlikely that this was the sole determinant for avirulence, as four of the 17 isolates tested originally were genotyped as virulent at the *AvrLm6* locus, but could not infect *B. juncea* cv. Aurea. Furthermore, deletion of the *AvrLm6* gene did not always correlate with the phenotype of progeny in either of the crosses described above. These data suggested that a second gene was involved in conferring avirulence to *B. juncea*. Mutations were also detected within two genes linked to *AvrLm6* in the *juncea*-attacking isolates: *LmCys1*, which had a single nucleotide polymorphism (SNP) leading to an amino acid substitution, and *LmCys2*, which was deleted. Complementation of IBCN18 with *LmCys2* did not confer avirulence (data not shown). *LmCys1* has been tested previously in complementation experiments and shown not to confer avirulence towards *Rlm6* (Fudal *et al.*, 2007).

The *juncea*-attacking isolate, IBCN18, was then crossed with a nonattacking isolate, 04P013. Both of these isolates are virulent towards cultivars with *Rlm6* (i.e. have the virulent allele at the *AvrLm6* locus), thus allowing mapping of the second avirulence locus. Of the 69 random progeny generated, 38 were avirulent and 31 were virulent on *B. juncea* cultivars Aurea and Stoke ($\chi^2 = 0.71$, $0.5 > P > 0.3$). This 1:1 ratio confirms the presence of a second gene, termed *AvrLmJ1* hereafter, responsible for conferring the

phenotype of *B. juncea* avirulence. Molecular markers were screened against the 69 progeny and a linkage map was developed. Five markers on Super Contig 7 formed a linkage group (LOD scores > 3.0) with the avirulence phenotype (*AvrLmJ1*) (Fig. 3), with markers MinLm1139 and MinLm1377 flanking the locus.

To identify candidate genes for *AvrLmJ1*, previously generated RNA-seq data were mined to identify genes in the mapped region of Super Contig 7 encoding small secreted proteins that were highly expressed *in planta*. RNA was isolated from cotyledons of cv. Westar, 7 days after inoculation with isolate IBCN18, and also from an *in vitro*-grown culture. RNA-seq was carried out, sequence reads were aligned to the reference *L. maculans* genome sequence (that of isolate v23.1.3), which does not attack *B. juncea*, and expression values were calculated as fragments per kilobase of exon per million mapped reads (FPKM). Within 215 kb of the mapped region, only one gene (Lema_uP070880.1; GenBank accession number XP_003834945) was predicted to encode a small secreted protein (Fig. 4A). This intronless gene was 57 and 35 kb from the closest genes, and was much more highly expressed (1400 FPKM) *in planta* than were the other genes in this region. Furthermore, the level of expression was 5380 times higher *in planta* than in *in vitro* culture (data not shown).

Further analysis of the RNA-seq data showed an alternative interpretation of the translation of Lema_uP070880.1; another ATG was located 201 bp upstream of the previously published start site. The newly predicted protein was 141 amino acids in length, including cysteine residues, and had a secretion signal (Fig. 4B). We hereafter refer to this gene as Lema_uP070880.2 (GenBank accession number KF853561). BLAST searches did not reveal sequence similarity with any reported genes. This gene was embedded in an AT-rich region (68.1% and 69.5% AT in 3 kb upstream and downstream, respectively). The open reading frame itself was 55.6% AT.

Lema_uP070880.2 was sequenced from the 16 isolates that had been screened for virulence towards *B. juncea* cultivars. Four polymorphic sites were identified within the 16 isolates, giving rise to five different alleles (Fig. 4B; Table 3). The allele from the reference genome of isolate v23.1.3 was defined as Lema_uP070880.2_0. Three of the polymorphic sites (R³⁸L, K⁵⁵R, K⁵⁵T) were identified in the avirulent isolates, whereas the fourth

Fig. 2 Complementation of *juncea*-attacking *Leptosphaeria maculans* isolate, IBCN18, with *AvrLm6*. (A) Average pathogenicity scores (from 32 inoculation points) of four transformants of isolate IBCN18 complemented with *AvrLm6*. Error bars represent the standard deviation of the mean pathogenicity scores. (B) Representative cotyledons of either *Brassica napus* cv. Westar or *B. juncea* cv. Aurea. Isolate IBCN18 is virulent on both cultivars, whereas the transformants are avirulent on *B. juncea*.

polymorphism, R²⁹Stop, was only identified in IBCN18 and V4, the two *juncea*-attacking isolates (Table 3, Fig. 4). This presence of the premature stop codon would result in a truncated protein of only 10 amino acids after processing of the signal peptide. Sequencing of a further 62 isolates did not reveal any additional alleles, with

the Lema_uP070880.2_0 allele being the most prevalent (Table 3).

The polymorphism conferring the premature stop codon in isolate IBCN18 conferred a restriction fragment length polymorphism (RFLP), whereby *Av*II cuts in the wild-type allele of isolate

Fig. 3 Mapping the avirulence gene, *AvrLmJ1*, of *Leptosphaeria maculans*. Sixty-nine progeny of a cross between the *Brassica juncea* attacking isolate, IBCN18, and nonattacking isolate, 04P013, were generated and screened for avirulence (*AvrLmJ1*) towards *B. juncea* cultivars Aurea and Stoke, and also with mini- and microsatellite markers. The *AvrLmJ1* gene is linked [logarithm of the odds (LOD) score > 3.0] to markers on Super Contig 7 of the genome. The distance (cM) for each marker from the *AvrLmJ1* locus is shown.

04P013, but not in allele 4, in which a premature stop codon is introduced (in IBCN18). A 479-bp region including the open reading frame was amplified from all 69 progeny of the IBCN18 and 04P013 cross. After digestion with *Avall*, a single band (479 bp) indicated the IBCN18 allele (premature stop codon), whereas two bands (118 and 361 bp) indicated the 04P013 allele. There was a 100% correlation between the presence of the premature stop codon and the virulence phenotype on *B. juncea*.

An avirulent allele of Lema_uP070880.2 was then transformed into isolate IBCN18. A DNA fragment (1819 bp) containing the coding region (426 bp), together with up- and downstream regions (1021 and 372 bp, respectively), was PCR amplified from genomic DNA of isolate v23.1.3 using primers with restriction sites on the ends (Table 2). This fragment was cloned into plasmid pZP-Nat using *Bam*HI and *Stu*I (Elliott and Howlett, 2006). The resulting plasmid was transformed into isolate IBCN18. All five nourseothricin-resistant transformants tested had become avirulent on *B. juncea* cultivars Aurea, Stoke and Forge, but were virulent on cv. Westar (Fig. 5). These experiments confirmed that the Lema_uP070880.2_0 locus conferred avirulence towards *B. juncea* cultivars and therefore was the mapped *AvrLmJ1* gene. Although SNPs were detected within the coding region of *AvrLmJ1*, isolates with these SNPs conferred an avirulence phenotype towards the *B. juncea* cultivars. Accordingly, only the Lema_uP070880.2_0 allele was used for complementation.

This *AvrLmJ1* gene shows the typical characteristics of *L. maculans* avirulence genes, such as location within a gene-poor region, containing multiple cysteine residues, and a predicted secretion signal. However, unlike the other *L. maculans* avirulence genes, the mutation conferring virulence within the *AvrLmJ1* gene is a single polymorphism leading to a premature stop codon. Deletion of the entire avirulence gene is the major mechanism for conferring virulence towards *Rlm1* and *Rlm6* in *L. maculans* populations (Fudal *et al.*, 2007; Gout *et al.*, 2007; Van de Wouw *et al.*, 2010). Virulence towards *Rlm4* is conferred by a single amino acid substitution, whereas virulence towards *Rlm7* is conferred by deletion of the avirulence gene or by RIP mutations (Daverdin *et al.*, 2012; Parlange *et al.*, 2009; Van de Wouw and Howlett, 2012). Multiple stop codons generated by RIP mutations confer virulence within the *AvrLm4-7* and *AvrLm6* alleles. Although RIP mutation has been reported to affect multicopy DNA, this process is leaky and can mutate adjacent single copy genes (Van de Wouw *et al.*, 2010). Intriguingly, in spite of *AvrLmJ1* being surrounded by repetitive DNA, the stop codon did not appear to be generated by RIP; indeed, no RIP-like mutations were detected within the gene.

The resistance gene in *B. juncea* complementary to *AvrLmJ1* is unknown. Blackleg resistance in *B. juncea* is poorly understood. Two strategies have been used to identify resistance genes in this species. The first involves introgression of resistance genes from *B. juncea* into *B. napus*, followed by analysis of segregation of resistance in *B. napus* (Chevre *et al.*, 1997; Roy, 1984). These researchers showed evidence for the presence of three dominant resistance genes in *B. juncea*. Two have been named *Rlm5* and *Rlm6*, both of which have been introgressed individually into *B. napus* to generate *Rlm6*-containing cultivars FalconMX and DarmorMX (Chevre *et al.*, 1997) and *Rlm5*-containing lines 150-2-1 and 151-2-1 (Balesdent *et al.*, 2002). Unfortunately, none of these cultivars/lines are available to us.

Using a second strategy, other research groups have analysed blackleg resistance by crossing two *B. juncea* lines (Christianson *et al.*, 2006; Keri *et al.*, 1997; Saal *et al.*, 2004). These groups identified the presence of a recessive resistance gene (*LMJR2*) in *B. juncea* in addition to a dominant gene (*LMJR1*); however, it is unknown whether *LMJR1* corresponds to *Rlm5* or *Rlm6*. Furthermore, Keri *et al.* (1997) showed that the recessive gene is epistatic to the dominant resistance gene.

These results are consistent with our finding that at least two avirulence genes, *AvrLm6* and *AvrLmJ1*, are involved in virulence towards *B. juncea*. Given the previous identification of both *Rlm5* and *Rlm6* in *B. juncea* and the data in this article, *AvrLmJ1* may correspond to *AvrLm5*. Alternatively, *AvrLmJ1* may correspond to the recessive resistance gene, *LMJR2*. However, as *Rlm5*-only lines are not available, we cannot test these hypotheses.

As a result of the presence of a recessive resistance gene, introgression of *B. juncea* resistance into *B. napus* would lead to

Fig. 4 Location, expression and predicted protein sequence of alleles of *Leptosphaeria maculans* *AvrLmJ1*. (A) The candidate gene, Lema_uP070880.1, is located within a gene-poor region of Super Contig 7, and is 57 and 33 kb from adjacent genes. RNA-seq data reveal that it is highly expressed *in planta*. FPKM, fragments per kilobase of exon per million mapped reads. (B) Lema_uP070880.2 (GenBank KF853561) encodes a small, cysteine-rich protein with a signal peptide (19 amino acids). Cysteine residues are highlighted with asterisks, amino acid polymorphisms are listed for each allele and X indicates the premature stop codon in allele Lema_uP070880.2_4. For allele 1, nucleotide changes resulting in K⁵⁵R are AAA to AGA. For allele 2, nucleotide changes for K⁵⁵T are AAA to ACA. For allele 3, nucleotide changes resulting in R³⁸L and K⁵⁵R are CGG to CTG and AAA to AGA, respectively. For allele 4, nucleotide changes resulting in R²⁹ Stop, R³⁸L and K⁵⁵R are CGA to TGA, CGG to CTG and AAA to AGA, respectively.

Table 3 Allele frequencies of Lema_uP070880.2.

Allele	Isolates (frequency)	Number of nucleotide changes	Coding sequence change	Phenotype on <i>B. juncea</i> cultivars
Lema_uP070880.2_0	34 (43%)	N/A	N/A	Avirulent
Lema_uP070880.2_1	18 (23%)	1	K ⁵⁵ R	Avirulent
Lema_uP070880.2_2	15 (19%)	1	K ⁵⁵ T	Avirulent
Lema_uP070880.2_3	9 (12%)	2	R ³⁸ L, K ⁵⁵ R	Avirulent
Lema_uP070880.2_4	2 (3%)	3	R ²⁹ Stop, R ³⁸ L, K ⁵⁵ R	Virulent

The sequenced isolate, v23.1.3, has the Lema_uP070880.2_0 reference allele; isolates IBCN18 and V4 have the Lema_uP070880.2_4 allele, which has a premature stop codon.

N/A, not applicable.

the transfer of only the dominant resistance gene, whereas the recessive gene would be lost, assuming that this recessive gene is absent in *B. napus*. This is consistent with the low frequency of the virulence allele of *AvrLmJ1* detected in the 86 isolates collected from 1986 until 2011; the virulent allele was only detected in the two *juncea*-attacking isolates collected prior to the 1990s. Further-

more, these data are consistent with *AvrLmJ1* conferring species-specific rather than cultivar-specific avirulence, as the recessive gene may be present in all *B. juncea* cultivars. This report also highlights the usefulness of RNA-seq data to refine gene models and to detect upregulation *in planta*, a characteristic of fungal avirulence genes.

Fig. 5 Complementation of the *juncea*-attacking isolate, IBCN18, with Lema_uP070880.2. (A) Average pathogenicity scores (from 32 inoculation sites) of five transformants of isolate IBCN18 complemented with Lema_uP070880.2_0. Error bars represent the standard deviation. (B) Representative cotyledons of either *Brassica napus* cv. Westar or *B. juncea* cv. Aurea. Isolate IBCN18 is virulent on both cultivars, whereas the transformants are avirulent on *B. juncea*.

ACKNOWLEDGEMENTS

We thank the Grains Research and Development Corporation for funding, the Victoria Life Sciences Computation Initiative for com-

putational resources via grant #RAS 990, the University of Melbourne for an Early Career Researcher award for R.G.T.L., and Dan Cross of Agriculture and Agri-Food Canada for seed of *B. juncea* cv. Forge.

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