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ORIGINAL ARTICLE

A new set of international *Leptosphaeria maculans* isolates as a resource for elucidation of the basis and evolution of blackleg disease on *Brassica napus*

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Abstract
A collection of isolates of the fungi *Leptosphaeria maculans* and *L. biglobosa*, which cause blackleg disease on *Brassica napus* (canola/oilseed rape) and other *Brassicaceae* species, was assembled to represent the global diversity of these pathogens and a resource for international research. The collection consists of 226 isolates (205 *L. maculans* and 21 *L. biglobosa*) from 11 countries. The genomes

Angela P. Van de Wouw and Jack L. Scanlan contributed equally to the paper.

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of all 205 *L. maculans* isolates were sequenced, and the distribution and identity of avirulence gene alleles were determined based on genotypic information and phenotypic reactions on *B. napus* lines that hosted specific resistance genes. Whilst the frequencies of some avirulence alleles were consistent across each of the regions, others differed dramatically, potentially reflecting the canola/oilseed rape cultivars grown in those countries. Analyses of the single-nucleotide polymorphism (SNP) diversity within these *L. maculans* isolates revealed geographical separation of the populations. This "open access" resource provides a standardized set of isolates that can be used to define the basis for how these fungal pathogens cause disease, and as a tool for discovery of new resistance traits in *Brassica* species.

KEYWORDS
avirulence gene, blackleg, canola, culture collection, oilseed rape

1 | INTRODUCTION

Leptosphaeria maculans and *L. biglobosa* are sister species that cause blackleg or phoma stem canker on *Brassica* species, leading to crop losses and major economic impacts, especially on canola (oilseed rape, *Brassica napus*) worldwide. These fungi cause a diverse set of symptoms, depending on the growth stage of the plant that they infect (Figure 1). Sexual spores (ascospores) are released from pseudothecia on infested canola stubble (crop debris). Once landed on the plant, the spores germinate and can enter via stomata or wounds, grow into the vascular tissue and then colonize the stem cortex, resulting in girdling of the stem (Hammond et al., 1985). The resulting severity of the disease differs for the two species, with *L. maculans* considered more damaging than *L. biglobosa*. Traditionally, *L. maculans* has been considered to infect cotyledons and lower leaves, which then allows the fungus to grow down into the stem and cause crown cankers, whilst *L. biglobosa* was initially considered to cause disease slightly later, leading to upper stem lesions (Fitt, Brun, et al., 2006; Fitt, Huang, et al., 2006). However, more recently *L. biglobosa* has been shown to heavily colonize the base of the stem in

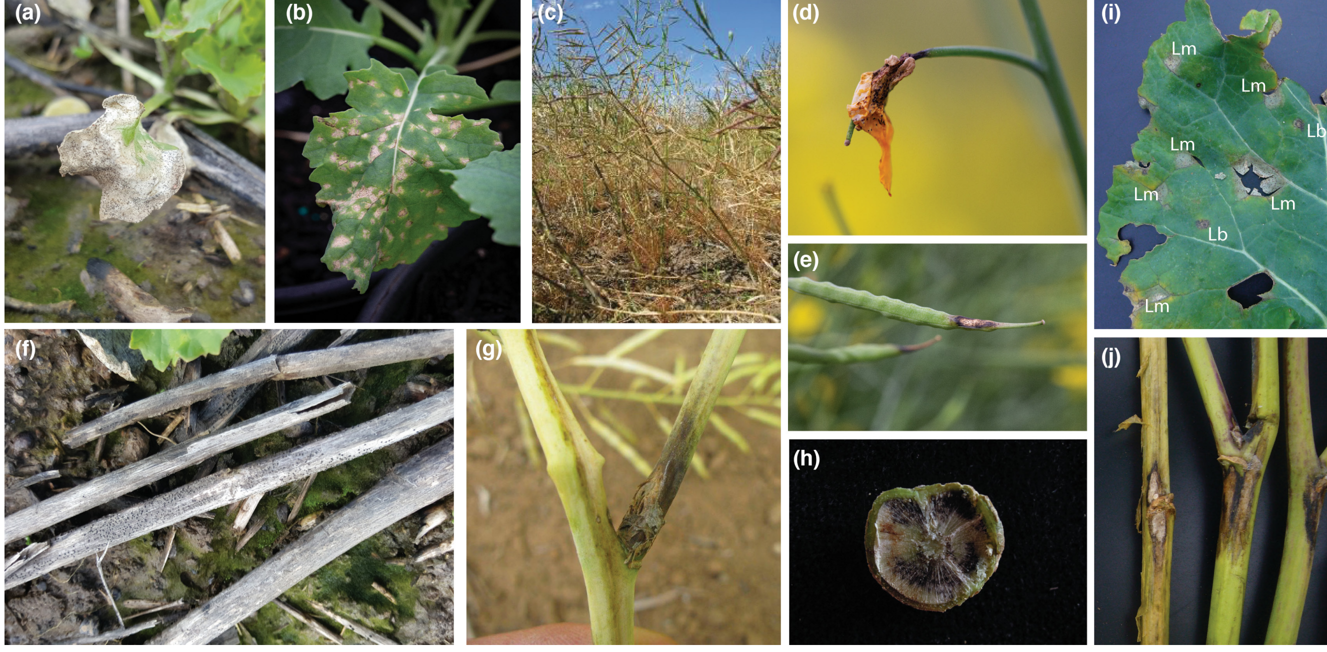

FIGURE 1 Symptoms of *Leptosphaeria maculans* (a–h) and *L. biglobosa* (i, j) on canola (*Brassica napus*). *L. maculans* is considered the more aggressive species and can infect cotyledons (a), leaves (b), flowers (d), branches (g) and pods (e). The pathogen colonizes the internal vascular tissue leading to crown cankers (h), which ultimately kill the plant (c). The fungus then remains within the stubble (crop debris) between growing seasons (f). Whilst *L. biglobosa* can colonize both the leaves (i) and upper stems (j), the lesions are smaller and less severe. Lm, *L. maculans*; Lb, *L. biglobosa*.

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both China and France, suggesting that this species can have significant impact on disease in some situations (Cai et al., 2018; Jacques et al., 2021). Recently in Australia, infection of the upper stems and branches by *L. maculans*, in addition to the traditional crown canker, has been observed (Sprague et al., 2018). Disease control relies on avoiding inoculum, applying fungicides and breeding for resistance, with the latter being the most universal approach to minimizing the disease.

The genetics of resistance to blackleg involves two different forms: qualitative (major gene) and quantitative (minor gene). Major gene resistance follows the concept of Flor's gene-for-gene interaction, wherein cultivars with particular resistance genes are resistant to disease if the fungal population carries the corresponding avirulence gene (Balesdent et al., 2005; Flor, 1955). To date, 16 major resistance genes have been characterized for the *L. maculans–Brassica* interaction, with five of these genes cloned (for a review see Borhan et al., 2022). Of the corresponding avirulence genes, 12 have been cloned and characterized, setting up the *L. maculans–Brassica* system as a model system for understanding host–pathogen interactions. In contrast, quantitative resistance is less well understood and is controlled by an unknown number of genes. This form of resistance restricts the colonization of the pathogen and therefore reduces the impact of the stem girdling (crown canker). Previously, quantitative resistance was thought to be expressed only in the adult stages of the plant and was generally effective against all isolates equally (Stuthmann et al., 2007). However, subsequent studies have shown that quantitative resistance is expressed throughout the life of the plant, and isolate-specific interactions occur (Marcroft et al., 2012; Schnippenkoetter et al., 2021). Whilst the molecular basis of quantitative resistance remains unclear, recent studies have shown that effector molecules produced by the fungus at late stages of the infection, as well as avirulence effector proteins, may also be involved in gene-for-gene interactions that can contribute to quantitative resistance (Gervais et al., 2017; Jiquel et al., 2021).

Developing a model system to identify and characterize fungal genes involved in blackleg disease has been challenging. Different criteria to classify isolates and different nomenclatures were used by research groups in different countries in the early 1990s when this pathogen was beginning to be analysed in some detail. Some research groups classified isolates as Tox^+ and Tox^0 by their ability to produce a phytotoxin, sirodesmin (Balesdent et al., 1992). Other research groups classified isolates as aggressive or non-aggressive, or as one of four pathogenicity groups (PG) based on their ability to infect three different *B. napus* cultivars (Balesdent et al., 2001). During this time, several molecular techniques (e.g., isozyme analysis, PCR, electrophoretic karyotyping and restriction fragment length polymorphism [RFLP] analysis) that could be used to characterize isolates had become available. In 1993, at the International Congress of Plant Pathology in Montreal, Canada, researchers established the International Blackleg of Crucifers Network (IBCN) and decided to develop and share a set of approximately 100 isolates that would be characterized in molecular terms and for their ability to infect *Brassica* cultivars whose resistance genes had been surmised (Mendes-Pereira et al., 2003; Rouxel & Seguin-Swartz, 1995). By 1995, this collection consisted of 93 isolates from France, Germany, Poland, Canada, the United States and Australia, recovered from *B. napus* (76), *Brassica juncea* (10) and weedy crucifers (7) (Balesdent et al., 2005). These isolates were maintained and distributed from Saskatoon, Canada and Versailles, France to members of the IBCN who then resolved to characterize them. Over the subsequent years, these isolates have been analysed using random amplified polymorphic DNA (RAPD) markers, amplified fragment length polymorphisms (AFLPs), electrophoretic karyotyping and more recently genome sequencing (Zander et al., 2013). Of the 93 isolates, 55 were *L. maculans*, 34 were *L. biglobosa* and four were different subspecies (Mendes-Pereira et al., 2003).

As genome sequencing became available, the entire genome of a reference isolate called v23.1.3, or more commonly JN3, was sequenced (Rouxel et al., 2011). This project revealed the complex bipartite nature of the *L. maculans* genome: a patchwork of GC-rich regions that contain the vast majority of the coding genes, and AT-rich regions that are riddled with remnant transposable elements. In 2018, the JN3 reference genome assembly was improved with the addition of Oxford Nanopore longread sequencing and refined gene annotations using RNA-seq data from diverse experimental conditions (Dutreux et al., 2018).

Establishing the genetics of blackleg resistance has not been straightforward. One challenge has been the complex nature of the genome of *L. maculans*. For instance, all of the avirulence genes identified to date are embedded in large repetitive and AT-rich regions of the genome, a location that makes sequencing particularly difficult, as illustrated by the efforts required to identify the first such gene, *AvrLm1* (Gout et al., 2006). Furthermore, some avirulence genes recognize more than one resistance gene. Another challenge has been in nomenclature and communication of results or resources. For instance, the same resistance gene in the plant or avirulence gene in the fungus has often been given different names by different research groups (Larkan et al., 2013; Long et al., 2011; Van de Wouw et al., 2009). Related to this challenge are implications of the complexity of the *B. napus* genome, which is derived from two diploid parents, resulting in the formation of an allotetraploid species. Thus, resistance genes may be derived from the different parents and may have homologues within one *B. napus* cultivar. The development of common resources and nomenclature is vital for understanding the basis of disease resistance because this knowledge is required for the commercial development of new sources of resistance by plant breeders.

The original IBCN collection has led to increased understanding of both the species structure of the blackleg pathogens and their interactions with host plants. However, there is a need for increased international efforts to ensure efficient and non-redundant blackleg research and for breeding for disease resistance in canola. Until now, most of the focus has been on gene-for-gene resistance, but as more information becomes available about quantitative resistance, the co-ordination and standardization of materials, methods and nomenclature becomes more imperative.

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Another important area that requires international collaboration is the study of the wide-scale population structures in these species. Scientists in individual countries have explored the diversity of *L. maculans* or *L. biglobosa* strains, dating back to the late 1990s (e.g., Badawy et al., 1991; Balesdent et al., 2005, 2022; Fernando et al., 2018; Liban et al., 2016; Mendes-Pereira et al., 2003; Mengistu et al., 1991; Van de Wouw et al., 2010). However, there is little understanding about the diversity and geneflow across countries. To address the challenges outlined above, in this study we describe the assembly and analysis of a set of isolates of *Leptosphaeria* spp. cultured from *Brassica* species across the globe.

2 | MATERIALS AND METHODS

2.1 | Culturing

Isolates were cultured from *Brassica* hosts using a range of methods, including isolation of ascospores from stubble, isolation of pycnidiospores from leaf lesions, stems or stubble or through in vitro genetic crossing. Once submitted to the collection, isolates were sent to the University of Melbourne, Australia, where strains were routinely cultured on 10% cleared V8 juice (Campbells) medium with 2% agar, supplemented with rifampicin (10 μg/mL) and chloramphenicol (30 μg/mL) to reduce risk of bacterial contamination. Isolates were grown at 22°C with a 12 h dark–light cycle and preserved either on 6 mm diameter cellulose discs kept dry with silica beads or as 15% glycerol stocks at −80°C. Isolate identification data, including year collected, location, *B. napus* line/cultivar the isolate was cultured from and isolation method, is noted in Table S1. Two in vitro isolates were included in the collection, IBCN094 (JN2) and IBCN095 (JN3), which were generated through artificial crossing, rather than being field isolates. These isolates have been used as wild-type reference isolates in many studies, with JN3 being the basis of the reference genome (Rouxel et al., 2011). These isolates were derived from crossing French and German isolates.

For production of mycelia for genomic DNA extractions, pycnidiospores were inoculated into 25 mL of 10% cleared liquid V8 juice and then cultured for up to 14 days. Mycelia were harvested using sterile Miracloth. For production of asexual spores for plant inoculations, isolates were cultured on 10% V8 juice agar for 10–14 days before plates were flooded with sterile water and scraped with a sterile scalpel blade to promote release of spores. The water/spore solution was filtered through Miracloth, adjusted to 5×10^6 spores/mL and stored at −20°C until used for plant inoculations.

2.2 | Whole-genome sequencing of isolates

Mycelia were freeze dried, after which genomic DNA was extracted using the DNeasy plant extraction kit (Qiagen) as per the manufacturer's instructions. DNA quantity was calculated using a NanoDrop spectrophotometer and quality examined by resolving on 1% agarose gels stained with ethidium bromide.

To confirm the species designations of the isolates, the internal transcribed spacer (ITS) regions of ribosomal DNA were amplified using primers PN3 (5′-CCGTTGGTGAACCAGCGGAGGGATC-3′) and PN10 (5′-TCCGCTTATTGATATGCTTAAG-3′) and conditions previously described (Mendes-Pereira et al., 2003). The ITS fragments were initially distinguished by size as *L. maculans* (555 bp) or *L. biglobosa* (580 bp) by separation of amplicons on 2% agarose gels. All amplicons identified as *L. biglobosa* were then sequenced using Sanger chemistry at the Australian Genome Research Facility (AGRF) and resulting sequences compared to published references on NCBI to determine the subspecies for each isolate.

The genomes of all *L. maculans* isolates were sequenced at the AGRF using 150 bp paired-end reads from NovaSeq sequencing. Libraries were prepared using Illumina DNA prep kit, as per the manufacturer's recommendations. The raw sequencing reads are deposited in GenBank under BioProject PRJNA902499, with SRA accessions, as well as genome statistics for each isolate in Table S2.

2.3 | Genome, single-nucleotide polymorphisms and population analysis

Reads were trimmed of adapters, overlapping pairs corrected, lightly quality trimmed (Phred > 5) and decontaminated of the phiX spike-in with BBMap v. 38.76 (Bushnell, 2022). Paired FASTQ files were converted to uBAM and assigned read groups using FastqToSam in GATK v. 4.2.5.0 (Poplin et al., 2018; Van der Auwera & O'Connor, 2020), converted back to interleaved FASTQ using GATK SamToFastq, and then mapped to the JN3 reference genome (Dutreux et al., 2018; GenBank: GCA_900538235.1) using BWA MEM v. 0.7.17 (Li, 2013). BAM files were compressed with Samtools v. 1.12 (Danecek et al., 2021) and then merged with the corresponding uBAM file using GATK MergeBamAlignment. PCR duplicates were marked with GATK MarkDuplicates; then, BAM files were reordered with GATK ReorderSam and indexed with GATK BuildBamIndex. GATK HaplotypeCaller was used to call variants (single-nucleotide polymorphisms [SNPs] and small indels) from individual samples, producing GVCF files using the flags '-ERC BP_RESOLUTION -ploidy 1 --assembly-region-padding 100'. GVCF files were sorted with GATK SortVcf, then joint genotyped using GATK GenotypeGVCFs.

For the population structure analyses, only SNPs with GATK SelectVariants were selected and then hard-filtered with the following filters in GATK VariantFiltration: 'QD < 20.0, QUAL < 50.0, SOR > 3.0, FS > 60.0, MQ < 40.0'. SNPs were further filtered in VCFtools to retain only biallelic SNPs that were present in at least two isolates and had a >90% genotyping rate. The set of filtered SNPs were LD-pruned in PLINK 1.9 (Chang et al., 2015) using the flag '--indep-pairwise' and a parameter of '100 1 0.1'. This created a window of 100 SNPs, calculated pairwise linkage disequilibrium (LD; r^2) within the window and removed one SNP from each pair if the pairwise LD > 0.1. The window was then shifted one SNP forward, and the process repeated.

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Two different analyses were used for analysing the population structure of the collection, principal component analysis (PCA) and genomic relationship matrix (GRM). For the PCA, PLINK 1.9 was used with the flag '--pca 30' to extract the top 30 principal components (PCs) (Table S3). The .eigenvec and .eigenval files were imported into R v. 4.2.2 for visualization with ggplot2 (Wickham, 2016). For the GRM, missing SNPs were imputed for each isolate using the median SNP value of the group (country). The resulting imputed data contained 41,856 SNPs from 205 isolates. Using the imputed data, the GRM was calculated following the method published by VanRaden (2008), which estimates genetic distances and evaluates genetic variability between populations. A heatmap of genetic distances was generated using R v. 4.2.2 (R Core Team, 2022). The PCA allowed an assessment of the variation across the multivariate dataset whilst GRM calculated the similarity of each pair of samples to generate a matrix.

2.4 | Characterization of avirulence alleles

For all cloned avirulence genes (*AvrLm1-LepR3*, *AvrLm2*, *AvrLm3*, *AvrLm4-7*, *AvrLm5-9*, *AvrLm6*, *AvrLm10*, *AvrLm11*, *AvrLm14* and *AvrLmS-LepR2*), genotypes were determined through analysis of the genome sequences. Known host × pathogen genetic interactions are as follows: *AvrLm1-LepR3* recognizes both *Rlm1* and *LepR3*; *AvrLm4-7* recognizes both *Rlm4* and *Rlm7*; *AvrLm5-9* recognizes both *Rlm5* and *Rlm9*; and *AvrLmS-LepR2* recognizes both *RlmS* and *LepR2* (currently uncharacterized R genes). Where possible, the identified alleles were compared to published genotypic data to infer phenotype based on the previous characterization of virulent and avirulent isolates (Balesdent et al., 2013; Fudal et al., 2009; Gout et al., 2007; Neik et al., 2022; Parlange et al., 2009; Plissonneau et al., 2016, 2017). The phenotypes for *AvrLm1*, *AvrLm2*, *AvrLm3*, *AvrLm4*, *AvrLm5*, *AvrLm6*, *AvrLm7*, *AvrLm9* and *AvrLepR3* were based solely on the genotype data because the avirulent and virulent alleles at these loci have been well characterized in the literature. For *AvrLm10*, *AvrLm11*, *AvrLm14* and *LmStEE98*, phenotypes of some alleles (e.g., repeat-induced point mutation [RIP] and deletion alleles) were inferred from genotype data; however, for the remaining alleles the phenotypes could not be determined due to limited information on correlations between genotype and phenotype and a lack of availability of lines with the corresponding resistance gene. For *AvrLmS-LepR2* and *AvrLepR1*, phenotypes were determined through screening on plants harbouring the corresponding resistance genes *LepR2* and *LepR1*, respectively. Avirulence alleles were named in accordance with the recent publication by Balesdent et al. (2022). All alleles identified by Balesdent et al. (2022) are described in a publicly available database (https://bioinfo.bioger.inrae.fr/portal/data-browser/public/leptosphaeria/avirulence_genes). Therefore, any new alleles identified as part of the current status were named using the same nomenclature and built on the existing database.

The late-effector *LmSTEE98* was also analysed as it appears to contribute to quantitative resistance, rather than to qualitative resistance (Jiquel et al., 2021). All alleles were named following the nomenclature description provided by Gautier et al. (2023) whereby different coding alleles (protein isoforms) are indicated by a different number, and different haplotypes of those alleles are designated a different letter.

To genotype *Avr* genes using short-read sequencing data, genomic loci (c.1 kb flanking the gene body) were extracted into separate GVCF files, and low-depth (DP < 3) and low-quality (GQ < 80) sites were removed using BCFtools view v. 1.15 (Danecek et al., 2021). The BCFtools consensus was used to generate FASTA files of each locus for each isolate, including confident SNPs and indels, and marking genotype-absent bases (removed in the previous step) with 'N's. Per-locus FASTA sequences for each isolate were aligned to the reference sequence using MAFFT v. 7 (Katoh & Standley, 2013), alignments were visualized in AliView (Larsson, 2014) and genotype variants were called relative to the reference. Alleles were considered deletions if Ns (due to low depth) spanned >90% of the locus, but these putative deletions were not independently validated with PCR or targeted sequencing.

The avirulence gene *AvrLepR1* is yet to be cloned, and therefore, the virulence of each isolate was determined by inoculation on Topas-LepR1, a line harbouring the corresponding resistance gene, *LepR1* (Larkan et al., 2016). In addition, all isolates were screened for virulence towards Topas-LepR2. This line was screened due to discrepancies in the literature regarding the genetic basis of its resistance. This includes issues with repeatability of screens and limited information on the relationship between genotype and phenotype.

For all in planta assays, the cotyledons of eight seedlings from each of the *B. napus* lines were wounded with a needle and inoculated with pycnidiospores of the *L. maculans* strains 10–14 days after planting. In addition to Topas-LepR1 and Topas-LepR2, Topas-DH15616 was used, as it is a universal susceptible control. Disease symptoms were recorded 10–17 days after inoculation and scored using the 0–9 scale as previously described (Koch et al., 1991; Van de Wouw et al., 2009). Isolates causing average pathogenicity scores of <3 were considered avirulent, scores ranging from 3 to 5 were considered intermediate, and scores of >5 were considered virulent.

3 | RESULTS

3.1 | Assembly of a set of *Leptosphaeria* strains representing current international diversity

A set of 226 *Leptosphaeria* isolates from 11 countries—Argentina, Australia, Canada, Czech Republic, France, Germany, Iran, New Zealand, South Africa, United Kingdom and the United States—was established (Table 1, Figure 2). The isolates have depositor names, and for convenience the numbering continues from the previous International Blackleg of Crucifers Network (IBCN) number, from IBCN094 through to IBCN330. Note that the new collection uses constant 3-digit identifiers—for example, IBCN099—instead of 1-to-3-digit identifiers—for example, IBCN18. Of these isolates, 205 were

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TABLE 1 *Leptosphaeria maculans* and *L. biglobosa* isolates contributed to the new International Blackleg of Crucifers Network (IBCN) database.

Country	Total number of isolates (Lm, Lb)[a]	Years represented	State/province/region represented[b]
Argentina	5 (5, 0)	2019	Buenos Aires
Australia	25 (25, 0)	2005–2006, 2012–2014, 2016, 2018–2020	New South Wales, South Australia, Victoria, Western Australia
Canada	19 (19, 0)	2009, 2012, 2015, 2018	Alberta, Manitoba, Saskatchewan
Czech Republic	8 (8, 0)	2017, 2019, 2020	Central Bohemia, Hradec Králové, Karlovy Vary, Liberec, Olomouc, Opava
France	20 (15, 5)	2012–2013, 2017–2019	Brittany, Bourgogne-Franche-Comté, Centre-Val de Loire, Hauts-de-France, Ile de France, Occitania
Germany	19 (19, 0)	2018–2020	Lower Saxony, Mecklenburg-West Pomerania, Schleswig Holstein
In vitro	2 (2, 0)	N/A	Generated through in vitro crossing between European isolates
Iran	18 (5, 13)	2017, 2021	Golestan, Mazandaran
New Zealand	28 (28, 0)	2011–2013	Canterbury, Hawke's Bay, Southland
South Africa	34 (34, 0)	2020–2021	Western Cape
United Kingdom	23 (20, 3)	2002–2003, 2010–2011, 2016–2017	Cambridgeshire, Hampshire, Hertfordshire, Norfolk, North Yorkshire, Nottinghamshire, Oxfordshire, Suffolk, West Yorkshire
United States of America	25 (25, 0)	2004–2007, 2014, 2016–2018	Georgia, North Dakota

[a]Lm, *Leptosphaeria maculans*; Lb, *L. biglobosa*.

[b]Specific locations from where isolates were cultured are presented in Figure 2.

identified as *L. maculans* and 21 as *L. biglobosa*. Detailed information about these strains is in Table S1. The growth of all isolates was examined in vitro to rule out any general growth defects indicative of overall fitness issues. All cultures grew and sporulated on 10% V8 agar, although colouration and growth patterns varied. Some of this variation is captured within Figure 3.

Whilst this project was designed to capture the genetic diversity of the fungi that cause blackleg disease on canola, the collection focused on *L. maculans*, due to its substantial impact on canola and oilseed rape crops. Although China is a major producer of canola, *L. maculans* has not been reported from China, only *L. biglobosa* (Deng et al., 2022). Due to export restrictions, no *Leptosphaeria* isolates were contributed from China to the collection.

For all countries, except Iran, *L. maculans* was the dominant species contributing to the collection. Of the 18 Iranian isolates, only five isolates were *L. maculans*, with the remaining 13 being either *L. biglobosa* 'canadensis' or *L. biglobosa* 'brassicae' (Table S1). The low proportion of *L. maculans* isolates contributed from Iran to this collection is consistent with other recent studies (Zamanmirabadi et al., 2022).

This collection is housed at the University of Melbourne and at the CBS-KNAW (Westerdijk Institute) culture collection (Table S1).

3.2 | Genome sequencing of isolates

Total sequences obtained for each isolate using Illumina short-read technology ranged from 3.6 million to 19.9 million read pairs (median: 8.1 million read pairs) representing between 1.09 and 5.96 Gb (median: 2.44 Gb). This corresponded to a theoretical mean coverage (against the reference genome size of 45.99 Mb) of 23.8–129.5× (median: 53×); however, after read preprocessing, mapping to the JN3 reference genome and marking PCR duplicates, the actual mean coverage ranged from 10.9× to 93.7× (median: 47.5×). Breadth of coverage across the genome ranged from 89.3% to 96.6% (median: 94.6%) at a depth ≥1, from 80.3% to 96.3% (median: 92.8%) at a depth of ≥3 and from 37.2% to 95.8% (median: 88.3%) at a depth of ≥10. The isolate with highest breadth of coverage at all three depth thresholds was the reference isolate JN3 (IBCN095), as expected. The absence of perfect (100%) breadth of coverage in the JN3 sample may highlight the challenge of mapping short reads to some highly repetitive parts of the *L. maculans* genome. The approximate 1%–2% gap between JN3 and the isolate with the next highest coverage breadth suggests the presence of unique sequence or complex structural variation in JN3 not found in other isolates in the collection. Genome coverage statistics for each isolate can be found in Table S2.

3.3 | Distribution of mating types

L. maculans is a heterothallic species: crossing between *MAT1-1* and *MAT1-2* isolates generates pseudothecia on stubble, in which ascospores develop. Across the entire collection, the mating-type alleles are at equal proportion (48.8% *MAT1-1* and 51.2% *MAT1-2*, $\chi^2 = 0.1$, $0.5 < p < 0.75$). However, this is not the case for each of the

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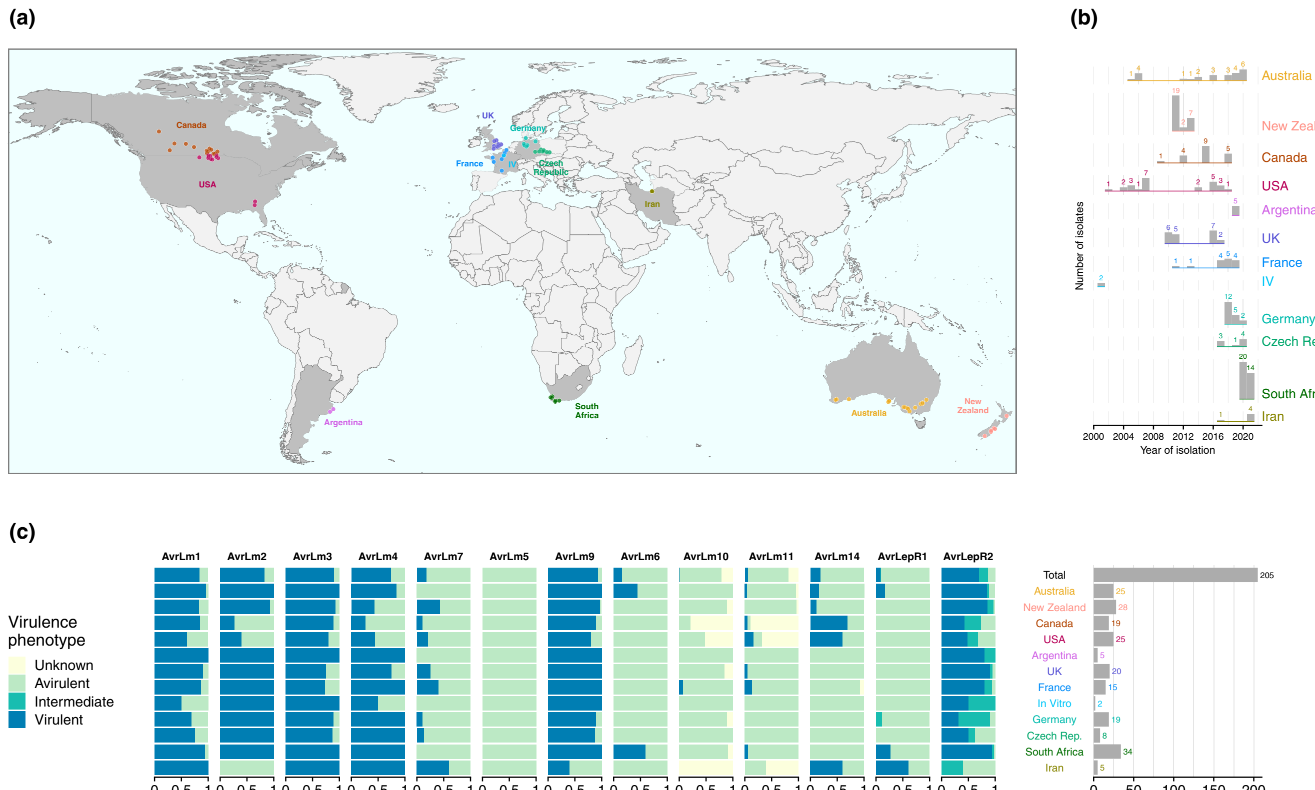

FIGURE 2 Location of isolates and avirulence allele frequency data from the *Leptosphaeria maculans* international isolate collection. (a) Locations of collection sites for individual isolates from the collection. (b) Breakdown of the number of isolates and year of collection for each country. (c) Frequency of virulence phenotypes for each of the cloned avirulence genes for each country.

individual countries. Isolates collected from Canada (13 *MAT1-1*, 6 *MAT1-2*) and the Czech Republic (1 *MAT1-1*, 7 *MAT1-2*) differed from a 50% ratio of the two mating types but this is probably due to the small number of isolates for these two countries.

3.4 | Genetic relationships between isolates

PCA suggested *L. maculans* isolates in the collection form three major groupings: European (UK, France, Germany, Czech Republic and 'In Vitro') and Argentinian isolates; Oceanic (New Zealand and Australia) and South African isolates; and North American (United States and Canada) and Iranian isolates (Figure 4). Most European isolates are tightly clustered with substantial overlap between countries, suggesting gene flow between the UK, France, Germany and the Czech Republic. Oceanic isolates separate neatly between Australia and New Zealand, with South African isolates near-evenly split in clustering with each country, perhaps indicating at least two origins of South African isolates from Oceanic countries. Three New Zealand isolates from Hawke's Bay were distinct from the rest of the country. North American isolates were less tightly clustered, with three outliers from the United States clustering with Europe; Iranian isolates were similar to United States and Canadian isolates.

Analysis of the genomic relationship matrix showed that the Australian isolates all clustered together, as did the South African isolates, whilst isolates from all other countries were distributed amongst clades (Figure 5). Interestingly, the New Zealand isolates collected from swede (rutabaga) were more similar to the European isolates than to the New Zealand isolates collected from canola. There was strong relatedness between the South African isolates and both the Australian and New Zealand isolates, with the overall heat map showing similar relatedness as the PCA results.

3.5 | Global distribution of avirulence (*Avr*) gene alleles

The 205 *L. maculans* strains were genotyped at *Avr* loci from gVCF files generated from the variant calling described above and/or phenotyped by disease symptom phenotypes on plants for their avirulence properties. For the avirulence genes that have been cloned, the genotypic diversity of each gene was determined (Table 2). For *AvrLm1-LepR3*, *AvrLm2*, *AvrLm4-7*, *AvrLm5-9*, *AvrLm6*, *AvrLm10A* and *10B*, *AvrLm11* and *AvrLm14*, low numbers of SNPs (ranging from 3 to 15) were detected, and as such, a low diversity of alleles was detected (between 3 and 13 plus deletion and RIP alleles). Conversely, *AvrLm3* and *AvrLmS-LepR2* had 28 and 31

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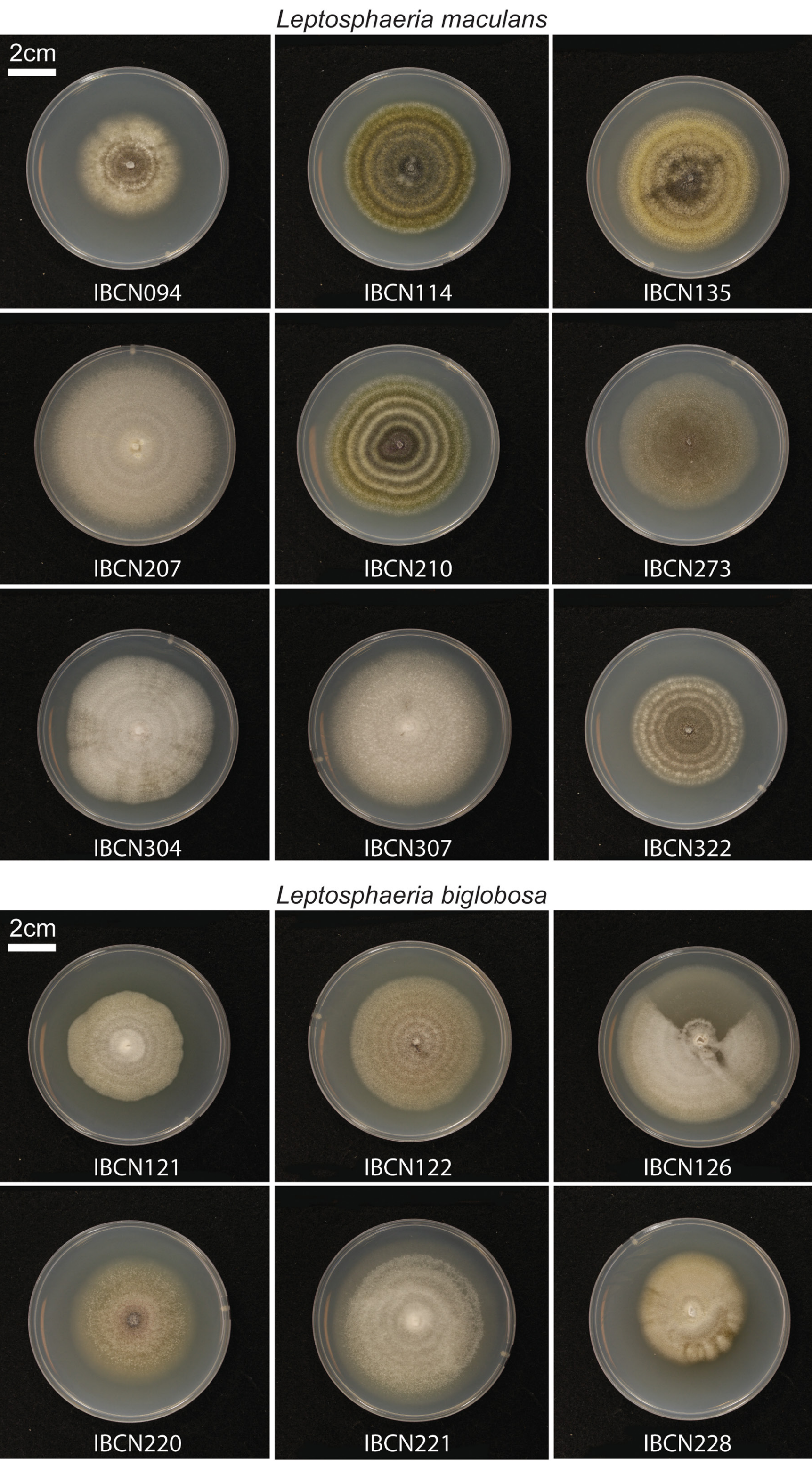

FIGURE 3 In vitro growth of *Leptosphaeria* isolates that were cultured on 10% cleared V8 juice agar at 21°C in a 12 h light/dark cycle for 16 days.

SNPs detected across the coding region, respectively, leading to 28 alleles (17 full-length protein variants) for *AvrLm3* and 35 alleles (12 full-length protein variants) for *AvrLmS-LepR2*. Deletion alleles were detected for all genes except *AvrLm5-9* and *LmStEE98*, ranging from 0.5% to 82.4% frequency, whilst RIP alleles were only found in six genes (*AvrLm1-LepR3*, *AvrLm2*, *AvrLm4-7*, *AvrLm6*, *AvrLm11* and *AvrLmS-LepR2*).

Across the worldwide collection, the frequency of alleles conferring avirulence to the corresponding *Brassica* resistance genes for the *L. maculans* loci *AvrLm1*, *AvrLm2*, *AvrLm3*, *AvrLm4* and *AvrLm9* was less than 30%, although for individual countries some of the gene frequencies varied substantially from the worldwide average (Figure 2). For instance, the frequency of *AvrLm2* was much higher in Iran (100%), Canada (74%) and the United States (60%) (Figure 2c).

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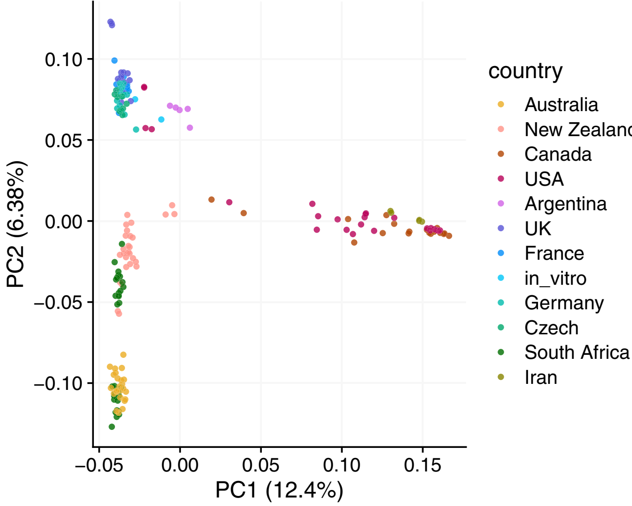

FIGURE 4 Principal component analysis of population structure of the international collection of *Leptosphaeria maculans* isolates. The analysis suggests *L. maculans* isolates form three major groupings: European (UK, France, Germany, Czech Republic and 'In Vitro') and Argentinian isolates; Oceanic (New Zealand and Australia) and South African isolates; and North American (United States and Canada) and Iranian isolates.

Whilst the frequency of avirulent alleles for *AvrLm6*, *AvrLm7*, *AvrLm10*, *AvrLm14* and *AvrLepR1* across the worldwide collection was >80%, these genes differed dramatically in specific countries. For example, avirulence frequency at the *AvrLm6* locus was 41% in South Africa, at the *AvrLm7* locus was 57% in New Zealand, at the *AvrLm14* locus was 31% in Canada and at the *AvrLepR1* locus was 40% in Iran. The frequency of avirulence at the *AvrLm5* locus was 100% across all populations (Figure 2c).

All isolates were screened for virulence towards resistance gene *LepR2*. The phenotype of the interaction between isolates with the *AvrLmS-LepR2* genotype and this resistance gene is not as clear cut as other gene-for-gene interactions reported (Liban et al., 2016; Neik et al., 2022; Van de Wouw et al., 2018). Isolates are classified into avirulent, intermediate or virulent classifications. In addition, the interaction phenotype varies depending on experimental conditions, as the interaction between identical isolates and *LepR2* seed lots were classified as virulent or avirulent, depending on the team and laboratory conditions (Neik et al., 2022). All isolates were initially screened in Australia. The frequency of avirulent isolates was low (14%) across the worldwide collection, and this was consistent across all countries (Figure 2c). When the phenotype data of each isolate were compared to the genotype data, there were inconsistencies. Isolates harbouring the deletion, RIP alleles or alleles encoding a truncated version of the protein (i.e., a stop codon within the coding sequence) were always virulent towards *LepR2*, as expected (Table 3). In addition to the deletion and RIP alleles, the four most prevalent alleles were *AvrLepR2_0*, *AvrLepR2_2*, *AvrLepR2_7* and *AvrLepR2_10*. For each of these alleles, isolates were identified with avirulent, intermediate or virulent phenotypes (Table 3) suggesting no correlation between genotype and phenotype. A subset of 12 isolates was selected and rescreened independently at BIOGER, France, to determine whether the results were consistent between screening environments. Isolate IBCN243, harbouring an RIP allele, was virulent when tested in both France and Australia and isolate IBCN108 was avirulent when tested in both locations. However, the remaining 10 isolates were either avirulent or intermediate on Topas-LepR2 when screened in France, and virulent when screened in Australia (data not shown).

The genotypic diversity was also assessed for *LmSTEE98*. Eight SNPs were detected at this locus, contributing to six different alleles (Table 2). The phenotypic consequences of these different alleles on disease severity are unknown.

4 | DISCUSSION

In the last few decades, the number of researchers studying *Leptosphaeria* in many countries worldwide has increased. This has led to a realization of the benefits of a well-characterized set of isolates, larger than the one developed in the 1990s. In this current study, a larger collection of isolates of *L. maculans* and a small number of isolates of *L. biglobosa* were developed, to form a basis for future research into blackleg disease of canola. Most of the discussion below is focused on *L. maculans* due to its importance as a pathogen and because of its abundance in canola-growing regions around the world.

Both mating types of *L. maculans* were identified in all geographic regions, suggesting that sexual reproduction can occur anywhere around the world. This observation has important implications for disease control, as mutation of avirulence genes or emergence of fungicide resistance will be able to spread through the population via sexual reproduction, potentially producing combinations of genotypes able to overcome current control measures used against blackleg disease. However, in two regions, the ratio of each mating type differed from 1:1, but this is probably due to the small number of isolates analysed in these countries. Whilst this is consistent with previous findings from Canada, it remains unclear why these ratios are skewed (Fernando et al., 2018; Zou et al., 2018).

Analysis of population structure across the collection can provide insight into the movement of isolates between regions. Although the number of isolates from several countries was small, non-random and non-systematic (with non-overlapping isolation dates between many countries), several findings were apparent. Whilst the analyses suggest there could be geneflow between regions, this might be partially restricted to within continents—for example, isolates from the UK, France, Germany and Czech Republic have high similarity, and isolates from the United States and Canada are similar. These data are consistent with previous studies based on a small number of minisatellite markers that discriminated populations from North America, Europe and Australia (Dilmaghani et al., 2012). Interestingly, Australia and New Zealand, whilst geographically closer to

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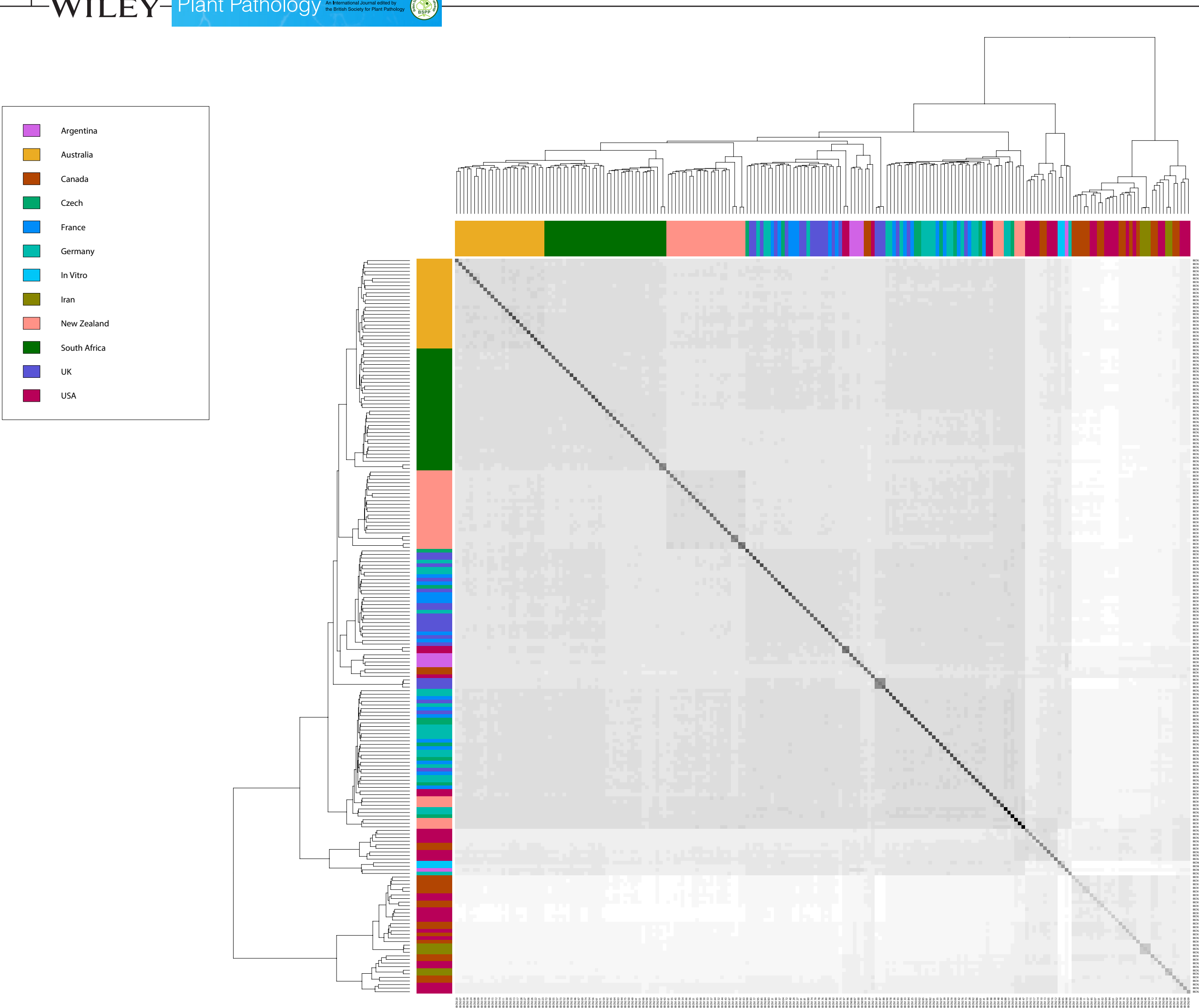

FIGURE 5 Analysis of the genomic relationship matrix using single-nucleotide polymorphisms across the genomes of the international collection of *Leptosphaeria maculans* isolates. The analysis shows similar groupings to that shown by the principal component analysis (Figure 4).

each other than the European and American continents, form distinct populations based on the SNP analysis, which suggests limited gene flow between them despite their close proximity. However, South African isolates show similarity to isolates from both Australia and New Zealand, yet are geographically further away. Similar continental groupings have been identified for other fungi such as the wheat pathogens *Parastagonospora* (*Phaeosphaeria*) *nodorum* and *Pyrenophora tritici-repentis* (Gurung et al., 2013; Stukenbrock et al., 2006) whereby limited or no subdivision was found between populations. Interestingly, the South African *P. nodorum* populations had low genetic diversity and the data suggested migration of isolates from Mexico as a founder population (Stukenbrock et al., 2006).

Whilst these general subpopulations are detected, there are exceptions: with Argentinian isolates grouping more closely with the European set of isolates and Iranian isolates grouping with North American isolates. Previously Argentina imported canola seed from Europe for many years and Iran has imported seed from Canada for the past two decades (Zamanmirabadi et al., 2022), which may have resulted in the similarities detected in the analysis. Furthermore, the population analysis shows that within New Zealand, isolates from Hawke's Bay on the North Island and the remaining isolates collected from the South Island are distinct, suggesting limited movement over a much smaller geographical distance. The GRM suggests that the isolates collected from swede (regardless of location) are more similar to European isolates than to isolates collected from canola within New Zealand.

Whilst there are similarities between some isolates from South Africa with those from either Australia or New Zealand, possibly suggesting contamination from these populations, South Africa imports seed from both Australia and Canada and yet no similarities were detected between South African and Canadian isolates. Historical trade routes that first introduced edible brassicas into Australia and New Zealand were via South Africa (Boshoff & Fourie, 2008, 2010). Furthermore, Argentina and Iran both predominately use Australian cultivars (Table 4), and yet there

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TABLE 2 Worldwide genotypic diversity of the characterized avirulence alleles of 205 isolates of *Leptosphaeria maculans*.

Avirulence gene	Number of alleles (and proteins) detected[a]	Additional deletion alleles detected[b]	Additional RIP alleles detected[c]	Number of SNPs detected[d]	SNP details	Amino acid changes[e]
AvrLm1-LepR3	3 (3)	Yes	Yes	4	297C>T, 423T>A, 493G>T, 512C>T	T83I, I125K, SYN, H155Y
AvrLm2	6 (5)	Yes	Yes	4	397A>G, 398A>G, 436C>G, 594C>G	H133G, Q146E, SYN
AvrLm3	28 (16)	Yes	No	31	6A>G, 15A>G, 22A>K, 49G>T, 50C>A, 67T>G, 75T>C, 105T>R, 135C>T, 142T>C, 152G>A, 168T>C, 171C>A, 172A>C, 173T>A, 195A>G, 228T>C, 232C>T, 234T>C, 243G>C, 253A>T, 255A>C, 313C>T, 380C>A, 391G>C, 396G>A, 397C>A, 401T>A, 404T>A, 439C>A, 459–461	SYN, SYN, I8V/F, A17S, A17E, C23G, SYN, D35E, SYN, SYN, S51N, SYN, S57R, I58L, I58H, SYN, SYN, L78F, SYN, SYN, I85L, SYN, H105Y, A127D, G131R, SYN, P133T, F134Y, I135K, R147S, D153Del
AvrLm4-7	13 (12)	Yes	Yes	12	94C>A 104A>G, 134T>C, 213G>C, 220G>A, 230C>T, 239T>C, 254G>T, 256G>A, 358G>C, 361C>A, 418C>T	R32S, Q35R, L45S, SYN, V74I, A77V, I80T, W85L, D86H, G120R, Q121K, intron
AvrLm5-9	6 (4)	No	No	4	6C>T, 85C>T, 113G>T, 164A>S, 426C>A	SYN, R38L, K55R/T, SYN
AvrLm6	5 (4)	Yes	Yes	4	4G>A, 7G>A, 513G>T, 578A>T	V2M, intron, G123C, Q144H
AvrLm10A	3 (1)	Yes	No	3	41G>A, 203T>C, 328C>A	SYN, SYN, SYN
AvrLm10B	4 (3)	Yes	No	3	329T>C, 332C>T, 530G>A	I110T, T111I, intron
AvrLm11	8 (5)	Yes	Yes	15	60C>T, 79C>T, 91G>A, 92A>C, 100A>G, 132C>T, 169C>T, 182A>G, 201C>T, 221G>A, 247C>T, 315G>A, 326T>C, 335T>C, 345G>A	SYN, Q27*, E31T, K34E, SYN, Q57*, N61S, SYN, R74Q, H82Y, intron, intron, SYN, E90K
AvrLm14	5 (2)	Yes	No	4	14C>T, 27C>T, 120T>A, 279T>G	T5M, SYN, SYN, SYN
AvrLmS-LepR2	35 (13)	Yes	Yes	28	3G>C, 30G>A, 35T>C, 39C>T, 42C>T, 49G>A, 50C>T, 51G>A, 57A>C, 70G>T, 70insA, 71del, 85C>T, 122G>A, 126G>A, 139G>A, 146C>T, 150A>T, 222G>A, 269C>T, 273C>A, 276T>G, 277G>A, 278G>A, 280G>A, 284G>A, 379G>A, 411C>T	M1I, SYN, F12S, SYN, SYN, A17T/I, SYN, Q24*, FS, FS, H29Y, R41Q, SYN, D47N, S49T, SYN, SYN, S90L, SYN, I92M, G93N/D, G94R, R95Q, G127S, SYN
LmSTEE98	6 (5)	No	No	8	9C>T, 34G>A, 50C>T, 64G>A, 79A>T, 105G>A, 190G>T, 198C>T	SYN, V12I, T17I, V22I, I27F, SYN, D64Y, SYN

[a]Number of alleles excludes RIP and deletion alleles. Number of proteins refers to only full-length proteins, therefore nonsense mutations such as frameshifts and stop mutations are excluded.

[b]Deletion alleles were characterized by low coverage of reads across the coding region of the gene. Deletion breakpoints were not defined and therefore all deletion alleles at a specific locus were treated as identical.

[c]Repeat induced point mutation (RIP) alleles were characterized by the presence of multiple G-C or A-T mutations within the coding region of the gene. Differences in RIP alleles were not determined and therefore all RIP alleles at a specific locus were treated as identical.

[d]SNP, single-nucleotide polymorphism.

[e] SYN, synonymous; *, stop codon; FS, frameshift.

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TABLE 3 Phenotypic variation of the different alleles at the *AvrLmS-LepR2* locus.

Allele	No. isolates with allele	Percentage of each phenotypic class (number)		
		Avirulent	Intermediate	Virulent
AvrLepR2_0	22	5 (1)	27 (6)	68 (15)
AvrLepR2_2	70	9 (6)	17 (12)	74 (52)
AvrLepR2_7	15	6 (1)	27 (4)	67 (10)
AvrLepR2_10	29	38 (11)	31 (9)	31 (9)
AvrLepR2_del	23	0 (0)	0 (0)	100 (23)
AvrLepR2_RIP	9	0 (0)	0 (0)	100 (9)

Note: All isolates from the collection were screened for virulence towards *LepR2* in Australia. Despite isolates having identical alleles, they produced different virulence phenotypes.

appears to be very limited similarity between the Iranian-Australian or Argentinian-Australian populations, at least given our limited sampling. Interestingly, there is no similarity between Canadian and Australian isolates despite an early speculation that blackleg might have been introduced to Canada from Australia (Buzza, 2007). The notion probably stemmed from the fact that blackleg epidemics occurred in Australia slightly earlier and there was canola germplasm exchange between the two countries during the 1970s. However, the result from the current study suggests otherwise. Collectively, these results suggest that movement due to contaminated seed may not be a common cause of geneflow.

An alternative hypothesis for the geneflow is that crop-growing practices and the genetic resistance in the cultivars being sown can lead to similar patterns of selection being placed on the population, thereby resulting in genetically similar patterns in different countries. In support of this, there are some similarities between the population analysis and the avirulence allele frequencies for each country: for example, the United States, Canada and Iran are the only regions with *AvrLm2* isolates and show high frequencies of *AvrLm14*. Consistent with this hypothesis, it is well documented that the resistance genes within a cultivar will influence the genetic structure of blackleg populations due to selection of isolates with the corresponding virulence allele (Rouxel et al., 2003; Zhang et al., 2016). This hypothesis could explain the similarities between the UK, Germany, France and the Czech Republic, whereby most regions are growing winter cultivars, which require vernalization, and that are predominately harbouring the effective resistance gene *Rlm7*, but that have also accumulated ineffective resistance genes used in the past like *Rlm1*, *Rlm2*, *Rlm4* or *Rlm9*. The similarities between South Africa and Australia could be due to growing the same cultivars (harbouring *Rlm6*) under very similar environmental conditions (spring type cultivars, sown in autumn, that is harvested in spring, canola–wheat–canola rotation, Table 4). However, the United States, Canada and Iran have very different cultural practices, and whilst the genetic resistance within cultivars sown in the United States is unknown, the resistance genes in Canada (predominately *Rlm3*) and Iran (predominately *LepR1*) vary (Table 4).

Across the worldwide population, the frequency of *AvrLm1*, *AvrLm2* and *AvrLm4* is generally low in all countries. This is consistent with the presence of *Rlm1* or *Rlm4* in many cultivars that are sown globally (Table 4). Interestingly, with the exception of the United States, Canada and Iran, the frequency of *AvrLm2* is less than 5% in all countries, which is consistent with previous allele frequency studies (Alnajar et al., 2022; Balesdent et al., 2023; Fernando et al., 2018; Stachowiak et al., 2006; Van de Wouw et al., 2018; Winter & Koopmann, 2016). Interestingly, for some countries, such as France, *Rlm2* has been used widely and probably contributed to the high frequency of virulence (Rouxel & Balesdent, 2017). However, in other countries such as Australia, *Rlm2* is not present in commercial cultivars (Van de Wouw et al., 2018). This raises the questions as to why the frequency of *AvrLm2* would remain low in almost all geographical regions. One possibility is that virulence at the *AvrLm2* locus has no or low fitness penalty and therefore can be maintained in the population once selection has occurred. Alternatively, a second, yet unidentified, resistance gene may also be recognizing *AvrLm2*, and this gene is present in most cultivars, leading to the continual selection of isolates virulent at the *AvrLm2* locus. Conversely to the situation with *AvrLm2*, the frequency of *AvrLm14* is much lower in Canadian, US and Iranian populations compared to the rest of the world. *Rlm14* was only described recently and was identified in American broccoli (*Brassica oleracea*) genotypes (Degrave et al., 2021). The higher levels of virulence in these populations may be linked to the presence of these broccoli varieties being sown in the United States.

Whilst many of the avirulence genes identified for the *L. maculans*–*Brassica* interaction have been well characterized, some have either proven difficult to work with, or there has been limited availability of isolates or plant material, and therefore, the understanding of virulence mechanisms are limited. An example of this is the *AvrLmS*–*LepR2* interaction. Whilst Neik et al. (2022) have identified the avirulence gene that is involved in the interaction with *LepR2*, the interaction between the plant and fungal partners does not appear to be straightforward. In the current study, there is limited correlation between genotype and phenotype. Whilst analysis of *AvrLmS-LepR2* from the isolate collection showed that RIP and deletion alleles lead to virulence, it remains unclear what other mechanisms may be responsible. Neik et al. (2022) found that expression levels of the *AvrLmS-LepR2* gene influenced the expression of the phenotype and therefore mutations within the up- or downstream coding regions may be responsible for the phenotype. Furthermore, the *AvrLmS-LepR2* and *LepR2* genetic interaction appears to be influenced by environment and therefore phenotyping may be challenging. The results from the current study show that, whilst some genotypes are consistent between different environments, others are not and therefore the data presented for this gene interaction,

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TABLE 4 Canola production systems across the world.

Country	Common resistance genes	Typical crop rotation	Cultivar phenology	Sowing time
Argentina	Generally unknown; however, many cultivars are Australian in origin and often harbour *Rlm6*	Wheat–canola–wheat	Spring	Autumn
Australia	Over 80% of Australian cultivars harbour *Rlm1*, *Rlm4* and/or *Rlm3*. *LepR1* and *Rlm6* are also present in c.30% of cultivars	Grown in rotation with wheat/barley/pulses. Frequency varies between 1 in 2 years to 1 in 4 years	Spring	Autumn
Canada	*Rlm3* is present in c.80% of Canadian cultivars. The next most common resistance gene is *LepR3* or *Rlm1*	Generally either canola–wheat–pulse or canola–wheat–canola	Spring	Spring
Czech Republic	Unknown; however, many cultivars are from French seed companies and probably have *Rlm7*	Usually oilseed rape–wheat–barley. Some areas also include cultivated corn, potato and other crops	Winter	Autumn
France	*Rlm7* is present in cultivars that account for 60%–70% of the acreage between 2013–2016. *Rlm3* is present in cultivars that account for an additional 20%–40% of acreage. *Rlm1*, *Rlm2*, *Rlm4* and *Rlm9* are defeated (ineffective) resistance genes but still present in cultivars accounting for 25%–65% of acreage	Oilseed rape is grown 1 in every 3 years. Rotation is generally with wheat or other winter-type cereals	Winter	Autumn
Germany	Unknown for most cultivars although *Rlm7* is present in some cultivars	Oilseed rape is generally grown 1 in every 3 years, canola–wheat–barley	Winter	Autumn
Iran	The most widely sown cultivar is Hyola50, from Australia, which contains *LepR1*. Other Australian cultivars are also sown	Wheat–canola–wheat rotation	Spring and winter	Spring types: autumn; winter types: end of summer
New Zealand	Generally unknown but *Rlm7* is present in some cultivars. The genetic resistance in swede cultivars is unknown	Generally grown every 2–3 years, but as few as 1 in 5 years. Usually in rotation with cereals, peas, potatoes, ryegrass/clover pastures or seed crops such as clover, ryegrass, linseed, radish, carrots. For swede, rotation with fodder beet, kale or ryegrass/clover pasture	Winter	Canola—mostly autumn sown; swede mostly spring sown for winter forage
South Africa	Over the past 10 years, *Rlm1* and *Rlm4* are present in c.60% of the cultivars. *LepR1* and *Rlm6* are in 10%–20% of cultivars. Many cultivars originate in Australia	Generally grown once in a four-year rotation, in rotation with cereals (wheat, barley, oats), lupin/peas, medic/clover pastures although shorter rotations (wheat–canola–wheat–canola)	Spring	Autumn
United Kingdom	Resistance genes *Rlm7* and *RlmS* are the most common in UK cultivars; recently some cultivars with a combination of *LepR1* and *Rlm7*	Grown 1 in every 5 years. Rotation is generally with cereals (wheat, barley, oats), beans/peas or sugar beet	Winter	Autumn
United States of America	Currently unknown	Predominantly wheat–wheat–canola; soybean–wheat–canola–wheat rotation	90% spring type; 10% winter	Spring types: spring; winter canola: autumn

in any study, must be interpreted carefully. However, without international isolate collections, such large-scale studies of genotype and phenotype would not be possible, and therefore, complex interactions such as between *AvrLmS-LepR2* and *LepR2* might be missed.

Currently, there is a range of nomenclature for both the *L. maculans* avirulence genes and the *Brassica* spp. resistance genes. This has originated from a number of sources: for instance, one avirulence product can be recognized by more than one resistance

gene (or even allele) by the plant; hence, in some cases the same avirulence factor was given multiple names (e.g., *AvrLmJ1*, *AvrLm5*, *AvrLm9*; Balesdent et al., 2005, Ghanbarnia et al., 2018; Plissonneau et al., 2018; Van de Wouw et al., 2014). A rationale for redeveloping an international isolate collection was to provide a common set of isolates that could help avoid nomenclature confusion wherein new resistance genes become available.

The naming of genes involved in qualitative resistance in the *L. maculans*–*Brassica* system is either *AvrLm#* or *AvrLepR#* in the pathogen, corresponding to *Rlm#* or *LepR#* in the host, with the *Lm* or *Lep* referring to *L. maculans*, as evident by all previous publications relating to these genes. It should also be noted that *L. maculans* was renamed *Plenodomus lingam* over a decade ago (de Gruyter et al., 2012), although this species nomenclature has not been taken up by researchers. One complication that has arisen in the literature is the identification of quantitative trait loci (QTLs) associated with quantitative resistance and the naming of these. To avoid confusion between qualitative and quantitative resistance, we are proposing that genes or QTLs associated with quantitative resistance be denoted *LmQR#*, making them easily distinguishable from qualitative resistance genes. Whilst no specific genes involved in quantitative resistance have been identified, multiple QTLs have been reported and this proposed nomenclature could be applied once the regions have been refined (for a review, see Amas et al., 2021). Furthermore, the use of the international isolate collection and associated genome, genotypic and phenotypic resources reported here should aid in the characterization of these QTLs, identification of the underlying resistance genes and consistent sharing of resources across the international community.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in GenBank BioProject at https://www.ncbi.nlm.nih.gov/bioproject/ with accession number PRJNA902499.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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