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1 **Breeding and management of major resistance genes to stem canker/blackleg in *Brassica* crops**

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13 **Abstract**

14 Blackleg (also known as Phoma or stem canker) is a major, worldwide disease of *Brassica* crop species
15 (e.g. oilseed rape, rapeseed, canola) caused by the ascomycete fungus *Leptosphaeria maculans*. The
16 outbreak and severity of this disease depend on environmental conditions and management practices
17 such as the use of fungicides and crop rotations, tilling, as well as a complex interaction between the
18 pathogen and its hosts. Genetic resistance is a major method to control the disease (and the only
19 control method in some parts of the world, such as continental Europe), but efficient use of genetic
20 resistance is faced with many difficulties: (i) the scarcity of germplasm/genetic resources available, (ii)
21 the different history of use of resistance genes in different parts of the world and the different
22 populations of the fungus the resistance genes are exposed to, (iii) the complexity of the interactions
23 between the plant and the pathogen, that expand beyond typical gene-for-gene interactions, (iv) the
24 incredible evolutionary potential of the pathogen and the importance of knowing the molecular
25 processes set up by the fungus to “breakdown’ resistances, so that we may design high-throughput
26 diagnostic tools for population surveys, and (v) the different strategies and options to build up the best
27 resistances and to manage them so that they are durable. In this paper, we aim to provide a
28 comprehensive overview of these different points, stressing the differences between the different
29 continents and the current prospects to generate new and durable resistances to blackleg disease.

30

31 **Keywords:** Blackleg; *Leptosphaeria maculans*; *Brassica*; genetic resistances; breeding

32 **The *Brassica* crop species**

33 The *Brassica* genus contains numerous crop species, including turnip and Asian cabbages
34 (*Brassica rapa*), broccoli, cauliflower and European cabbages (*Brassica oleracea*), rapeseed/oilseed
35 rape and swede (*Brassica napus*), and mustards (*Brassica nigra* - black mustard, *Brassica carinata* -
36 Abyssinian mustard, *Brassica juncea* - Indian mustard) for which centuries of domestication have
37 resulted in countless oilseed, leaf, root, and stem vegetable morphotypes. Related cultivated species
38 include *Sinapis alba* (white mustard), *Eruca sativa* (rocket), and *Raphanus sativus* (radish). These crops
39 have been present in the human diet since ancient times. For instance, Greeks reported the use of
40 mustard, cabbage, and kale in Europe before 0 CE, and in India *Brassica* crops first appeared around
41 2000 to 1500 B.C. (Bell 2012). The major crop species that are present in the genus *Brassica* comprise
42 three diploid species: *B. nigra* ($2n = 2x = 16 = BB$), *B. rapa* ($2n = 2x = 20 = AA$), and *B. oleracea* ($2n = 2x$
43 $= 18 = CC$), which hybridized to produce the allotetraploids *B. juncea* ($2n = 4x = 36 = AABB$), *B. carinata*
44 ($2n = 4x = 34 = BBCC$) and *B. napus* ($2n = 4x = 38 = AACC$) (U, 1935).

45 *Brassica napus* or rapeseed/oilseed rape/canola, the most economically significant crop in the
46 *Brassica* genus, appeared a few thousand years ago in *Brassica* cultivation areas (reviewed by Mason
47 and Snowdon, 2016) and is recorded as a crop in Europe in the early middle ages (Gupta and Pratap,
48 2007). Today, rapeseed is a crop used mainly for oil production, occupying the third place worldwide
49 after palm oil and soybean, with world production still increasing (FAO 2023). The major producing
50 regions are China, Canada, and Europe, with approximately 35 million hectares producing >76 million
51 tons in 2017, with yield increasing from 1.3 tons/ha in 1994 to 2.19 tons/ha in 2017. In Europe alone,
52 production increased from 1.55 tons/ha in 1961 to 2.81 tons/ha in 2021 (FAO 2023).

53 *B. napus* as a crop is vulnerable to numerous insect pests, but relatively few diseases are of
54 global importance: major, worldwide diseases include Sclerotinia stem rot, clubroot, and stem canker
55 (Zheng et al. 2020). Stem canker disease is caused by a complex of fungal species encompassing
56 *Leptosphaeria maculans* and *Leptosphaeria biglobosa*. Worldwide, stem canker disease is estimated
57 to cause annual losses of 10 - 15%, and in some cases up to 90% yield loss (Fitt et al. 2006; Sprague et
58 al. 2006; Zheng et al. 2020). As is commonly observed for many crop diseases, especially those with
59 aerial transmission, the outbreak of stem canker relies on a complex interaction between pathogen
60 species, host, climate, and cultural factors, such as the use of fungicides, use of resistant plants, crop
61 management, and rotations, physical distance between rapeseed crops, and accurate disease
62 forecasting (West et al. 2001; Van de Wouw et al. 2021). Therefore, in the search for more sustainable
63 rapeseed production, combinations of these strategies need to be applied to manage stem canker.
64 Among the different strategies, the selection and breeding of resistant varieties offers a promising
65 means to control stem canker disease incidence. The preferred control method is based on the use of

66 cultivars possessing one or more major resistance genes coupled with quantitative resistance, which
67 provides effective protection in a variety of environmental conditions (Delourme et al. 2006; Brun et
68 al. 2010; Huang et al. 2018).

69 *B. napus* is unfortunately highly inbred, limiting the availability of resistance sources within this
70 crop (Friedt et al. 2018). This is a result of its status as a recent allotetraploid species (originating from
71 hybridization between only a few progenitor genotypes) as well as a result of intensive breeding for
72 agronomic traits (Cowling 2007; Körber et al. 2012). No wild accessions of *B. napus* are known to exist
73 which can be accessed to improve the genetic diversity present in this crop (Dixon 2006). However,
74 the resynthesis of *B. napus* by crossing between varieties of the progenitor species *B. rapa* and *B.*
75 *oleracea*, as well as hybridization with sister allopolyploids *B. juncea* and *B. carinata* which share the A
76 and C genome with *B. napus* respectively, is one option to transfer useful traits and diversity, as is the
77 (more challenging) solution of hybridization with distant relatives (reviewed by FitzJohn et al. 2007;
78 Mason et al. 2010; Katche et al. 2019; Quezada-Martinez et al. 2021).

79 In summary, the management of the disease necessitates consideration of the following points:
80 the cropping cycle of the plant and its consequences on the fungal epidemiology, the fungal species
81 responsible for the disease, the available resistance sources and their use in the past, and the structure
82 and adaptability of pathogen populations. In all these aspects major differences exist across different
83 growing regions worldwide (Table 1) and the best resistance sources and their sustainable
84 management may differ from one continent to the other, rendering solutions for one situation
85 inapplicable for another.

86

87 **Table 1.** An overview of the characteristics of the disease due to *Leptosphaeria maculans* and the use
 88 of resistance genes in three major *Brassica napus* cropping areas where *Leptosphaeria maculans* is
 89 present

	Western Canada	Australia	France/Europe
Plant genotypes and cropping practices			
Plant genotypes grown	Spring types	Mostly spring types grown over winter	Winter types
Duration of the cropping season	3-4 months	5-7 months (11 months for “grain and graze” dual-purpose)	10-11 months
Specificities		Winter types grown as dual-purpose crops 100% herbicide-tolerant crops (mostly GM) Mostly hybrids	Mostly hybrids No GM cultivars, no herbicide resistance
Environmental hazards	Long and cold winter season	Drought	Frost
Stem canker/blackleg disease			
First identification of <i>L. maculans</i>	1969 (Petrie 1969); 1975 (at Saskatchewan) (Dolatabadian et al.2022)	1956 (Smith 1956)	1863 (Tulasne and Tulasne 1863)
First documented damages	1982 (at Saskatchewan) (Dolatabadian et al. 2022)	1972 (Roy and Reeves 1975)	1950s (Darpoux et al. 1957); 1964-1967 (Lacoste et al. 1969)
Epidemiology			
Primary inoculum	Ascospores and pycnidiospores (Zhang and Fernando 2018)	Ascospores	Ascospores

Importance of secondary inoculum	Common	Probably uncommon	Very low (West et al. 2001)
Duration of inoculum production	Just before and at flowering time (May-June)	Throughout the growing season (West et al. 2001)	Throughout the growing season (West et al., 2001)
Leaf spots	At flowering (June-July) (West et al. 2001)	Throughout the growing season (West et al. 2001)	Throughout the growing season (West et al. 2001)
Specificities of the disease	Seedling blight documented	Seedling blight documented Upper canopy infections (Amas et al. 2021)	No seedling blight
Survival of the fungus on residues	2-4 years	> 4 years	2-3 years (West et al. 2001)
Association with <i>L. biglobosa</i>	<i>L. biglobosa</i> 'canadensis'; common but with variability depending on location (Dilmaghani et al. 2009)	<i>L. biglobosa</i> 'canadensis', 'australensis', 'occiaustralensis': rare (Vincenot et al. 2008)	<i>L. biglobosa</i> 'brassicae': very common; systematic association of the two species (Jacques et al. 2020)
Control of the disease			
First documented use of resistance genes	1995 (<i>Rlm3</i>) (Zhang et al. 2016)	1975 (<i>Rlm4</i>) (Rouxel et al. 2003b; Roy and Reeves, 1975)	1970 (<i>Rlm2</i>) (Rouxel and Balesdent, 2017)
Other resistance genes used in commercial varieties	<i>Rlm1</i> or <i>LepR3</i> , <i>Rlm4</i> , <i>RlmS-Lep2</i> (Zhang and Fernando, 2018; Cornelsen et al. 2021)	<i>Rlm3</i> , <i>RlmS-Lep2</i> , <i>Rlm1</i> or <i>LepR3</i> , <i>LepR1</i> , <i>Rlm6</i> , <i>Rlm7</i> (Marcroft et al. 2012; Van de Wouw and Howlett 2020)	<i>Rlm1</i> , <i>Rlm3</i> , <i>Rlm4</i> , <i>Rlm7</i> , <i>Rlm9</i> , <i>RlmS-Lep2</i> , <i>LepR1</i> (Gautier et al. 2023 ; Balesdent & Jiquel, unpublished)
Breakdown of resistance gene	<i>Rlm3</i> : continuous between 1997 and 2010 (Zhang et al. 2016)	<i>LepR3</i> + <i>RlmS-Lep2</i> : 2003 (Sprague et al. 2006)	1972 (<i>Rlm2</i>); 1999 (<i>Rlm1</i>); c. 1999 (<i>Rlm4</i>) (Rouxel and Balesdent, 2017); 2022 (<i>Rlm7</i>) (Balesdent et al. 2022)

Successive/concomitant use of <i>Rlm</i> genes	Successive/Concomitant	Concomitant	Successive
Maintenance of defeated <i>Rlm</i> genes in commercial varieties	Yes	Yes	Yes
Knowledge of <i>Rlm</i> gene content in varieties	Yes	Yes	Yes (recently only)
Communication towards farmers on <i>Rlm</i> gene content	Yes: resistance groups with R gene content (Canola Council of Canada 2021)	No: resistance groups with no information on resistance genes (Van de Wouw and Howlett, 2020)	Yes: information on the presence of resistances showing efficiency (<i>Rlm3</i> , <i>Rlm7</i> , <i>RlmS-Lep2</i>) in each new variety (https://www.terresinovia.fr/columbia)
Use of fungicides	Common	Systematic (Van de Wouw et al. 2021)	Uncommon

90

91

92 **Biology and epidemiology of *L. maculans* and the *L. maculans*-*L. biglobosa* species complex**

93 Two *Leptosphaeria* species, *L. maculans*, and *L. biglobosa*, only discriminated in 2001
94 (Shoemaker and Brun 2001), are associated with stem canker disease (Rouxel and Balesdent, 2005).
95 One, *L. maculans*, is monomorphic, thought to be of recent origin (Dilmaghani et al. 2012), and present
96 in all *B. napus*-growing regions of the world, except China (Cai et al. 2018). The other, *L. biglobosa*, is
97 divided into many subspecies and present in all *B. napus*-growing regions with geographic specificities
98 (Mendes-Pereira et al. 2003; Vincenot et al. 2008). In many parts of the world, the two species are
99 found together in fields and individual plants at each stage of the disease (West et al. 2001; Jacques et
100 al. 2021). *L. biglobosa* is the only causal agent of the disease in China, where it has been suggested to
101 cause upper stem lesions and stem canker resulting in seed yield losses ranging from 10 to 37% (Cai et
102 al., 2018). In all other parts of the world, however, *L. biglobosa* is considered a minor contributor to
103 final yield losses compared to *L. maculans*. In addition, *L. biglobosa* has purely necrotrophic behavior
104 (Lowe et al. 2014), and no difference in susceptibility/resistance has been documented so far between
105 *Brassica* cultivars. Consequently, breeding for resistance has to date been targeted solely at *L.*
106 *maculans*, with evident successes impacting disease outcomes and reducing yield losses, at least over
107 short periods (Balesdent et al. 2022).

108

109 *History of the disease on different continents*

110 Early mycologists described fungal species on the basis of morphological criteria that differ
111 between the sexual and asexual forms of the species. This resulted in multiple distinct species names
112 for a single species and the need to scrutinize historical records to retrace the history of these fungal
113 species. For *L. maculans*, Tode in 1791 was the first to describe a fungal species associated with stem
114 diseases of cabbages, named at the time *Sphaeria lingam* (cited in Henderson, 1918). Subsequently,
115 the authors independently described both the asexually-reproducing stage of the fungus (the most
116 popular name being *Phoma lingam*) and the sexual stage (*L. maculans*). In France, *L. maculans* and *P.*
117 *lingam* were identified as two separate species as early as 1863 (Tulasne and Tulasne 1863). It was
118 only during the 20th century that experimental evidence that *L. maculans* and *P. lingam* are anamorphs
119 of the same fungal species was obtained in Europe and other parts of the world: in the 1950s in
120 Continental Europe (Müller and Tomasevic 1957), Australia and New Zealand (Smith 1956), and in the
121 1960s in the UK (Smith and Sutton, 1964) and Canada (Petrie 1969), thus substantiating the presence
122 of *L. maculans* in these parts of the world. The fungus was initially described on different crucifer
123 species, and the first damaging epidemics were described in cabbage in the USA (Henderson 1918).
124 However, it was only with the success of rapeseed as a major crop species, following years of breeding
125 for improved agronomic traits coupled with subsequent narrowing of genetic diversity, that the first
126 stem canker epidemics were recorded. As the first region to breed and grow rapeseed as a major crop

127 (1938 in France for example), continental Europe was the first region of the world to be impacted by
128 stem canker disease. The first epidemics were described in the 1950s in France (Darpoux et al. 1957)
129 and caused average yield losses of around 40% between 1964 and 1967 (Lacoste et al. 1969; Table 1).
130 In Australia, oilseed rape was first grown commercially in 1970 (in Western Australia), but in 1972,
131 blackleg impacted crop yields so severely that less than 15 500 ha were sown between 1974 and 1991
132 (Roy and Reeves 1975; Sivasithamparam et al. 2005). In Western Canada before the 1970s, the disease,
133 due to the sole presence of *L. biglobosa*, caused only very minor yield losses. *L. maculans* was first
134 identified in Saskatchewan in 1975 (McGee and Petrie 1978), and later in Manitoba, Alberta, and
135 British Columbia (Gugel and Petrie 1992). However, the first significant damages documented in
136 Saskatchewan were in 1982, with yield losses up to 56% in some fields (Dolatabadian et al. 2022; Table
137 1)

138

139 *Pathogenic cycle and epidemiology of L. maculans*

140 In Europe, the disease cycle starts with airborne ascospores produced on contaminated
141 stubble (West et al. 2001; Rouxel and Balesdent 2005; Table 1). These ascospores land on cotyledons
142 and leaves, where they germinate rapidly and the resulting hyphae penetrate the leaf through stomata
143 or wounds (West et al. 2001). If the plant is susceptible, the hyphae first grow slowly in the apoplast,
144 then massively colonize the intercellular spaces around the inoculation point to eventually cause
145 greyish-green leaf spots on which the asexual organs, the pycnidia, differentiate (West et al. 2001).
146 The pycnidiospores produced are of very limited importance in the disease cycle (at least in Europe)
147 due to continuous production of ascospores throughout the plant vegetative cycle, low dispersal ability
148 (rain-splash dispersal), and low infectivity of the pycnidiospores (Rouxel and Balesdent 2005).
149 Concomitantly with leaf spot development, the mycelium heads to the petiole and travels down the
150 stems asymptotically (Hammond et al. 1985; Huang et al. 2014). In the intercellular spaces of the
151 stem tissues, the fungus maintains low transcriptional activity for a few months, during winter, and
152 “revives” in spring with the initiation of waves of expression of genes associated with stem biotrophy
153 (Gay et al. 2021). Eventually, it turns necrotrophic again and expresses a stem base canker (“crown
154 canker”). In severe cases of stem canker, complete lodging and death of the plants occur (West et al.
155 2001). The fungus then lives as a saprobe on stem residues where it differentiates pseudothecia,
156 producing the sexual spores, and ascospores (West et al. 2001; Rouxel and Balesdent 2005).

157 In the three main growing regions of the world (excluding Asia, where no *L. maculans* has been
158 identified to date), the vegetative cycle of *B. napus* is different, explaining important differences in the
159 epidemiology/disease cycle of *L. maculans* between Europe, Western Canada and Australia (West et
160 al. 2001; Table 1). Western Canada exclusively grows spring-type varieties (canola) with a very short

161 vegetative cycle (3-4 months between May and August). These varieties have very few resistance
162 genes to *L. maculans* and a low R gene diversity (Rouxel et al. 2003a; Jiquel et al. 2021). At the other
163 extreme, Europe mainly grows winter-type rapeseed, necessitating vernalization to bloom, with a very
164 long (10 - 11 months) vegetative cycle, from early August to July (Table 1). These winter cultivars harbor
165 a limited variety of resistance genes (“R” genes) but, in contrast to spring varieties, genotypes devoid
166 of resistance genes to *L. maculans* are rare (Rouxel et al. 2003b). In Australia, the situation is more
167 complex, with mostly spring types grown for an extended time (5 - 7 months from March to
168 September), but also winter-types for dual-purpose used firstly for grazing livestock and then for seed
169 production. These latter types have a long vegetative cycle (11 months; Van de Wouw et al. 2021).

170 The *L. maculans* disease cycle is complex and strongly depends on the vegetative cycle of the
171 host plant. In particular, the pathogenic cycle has the specificity to necessitate an early entry point in
172 leaves, followed by systemic colonization of the plant stem, eventually resulting in stem canker a few
173 weeks before harvest. Thus, while leaf spots usually appear continuously throughout the growing
174 season (West et al. 2001) in Europe or Australia, they only are present at flowering time in Western
175 Canada, leaving a very short time for the fungus to proceed to the stem and develop the cankers which
176 are a prerequisite for differentiation of pseudothecia. In this respect, there are still debates in the
177 literature on the relative importance of pycnidiospores and ascospores to initiate and propagate the
178 disease in Western Canada, and leaf spots may be unnoticed before stem canker develops (Zhang and
179 Fernando 2018). This would be in accordance with similar findings on short vegetative cycle cabbages,
180 on which only pycnidia differentiate and clonality of the *L. maculans* population is the rule (Dilmaghani
181 et al. 2012). However, no signs of clonality have been identified in populations isolated from Western
182 Canada on canola (Dilmaghani et al. 2009), and the authors claim that pycnidiospores are important
183 contributors to the disease cycle in Australia (Li et al. 2007) or even the main inoculum source in
184 Western Canada (Zhang and Fernando 2018). However, further research is required to support this
185 claim with epidemiological studies.

186 In Europe, the stem canker is essentially a monocyclic disease (the secondary inoculum, pycnidiospores,
187 only have a very limited contribution to the epidemiology) (West et al. 2001). Pseudothecia
188 differentiate on leftover residues and remain present throughout the growing season, even for many
189 years (up to four in Australia), as long as the woody residues remain (West et al. 2001). This has
190 possible consequences in terms of quality and latency of inoculum production. In all cases, ascospores
191 are the primary inoculum, and are released under strict climate conditions requiring continuous
192 wetting of the residues by rain for maturation (Toscano-Underwood et al. 2003), and, at least in Europe,
193 a concomitant drop in temperature (Peres et al. 1996). Pseudothecial maturation is delayed by dry hot
194 summers in Western Australia and by sub-zero winter temperatures in Western Canada, allowing

195 synchronicity between maturation and sowing even though the delay between harvest and next
196 sowing is very long in Australia and even more in Western Canada (9 - 10 months after harvest) (West
197 et al. 2001). In Europe, mature pseudothecia can be observed at sites of severe cankers on stem bases
198 shortly after harvest (West et al. 1999). The period of ascospore release varies from region to region
199 but usually coincides with the presence of young, susceptible plants. In Australia and Canada, intense
200 and early ascospore release can cause seedling blight. Ascospores are then produced and released
201 throughout the growing season (Table 1), and there is a clear correlation between the intensity of
202 ascospore discharge and the number of leaf spots (Peres et al. 1996). Continuous ascospore discharge,
203 and changing practices such as early sowing, may also result in yield-limiting spotting on siliques (i.e.,
204 upper canopy infection), detected in Australia since 2010. While this symptom has been described
205 occasionally, e.g. on cabbage (Bonman et al. 1981), it is becoming a concern in Australia with
206 reductions in grain yield up to 30% (Van de Wouw et al. 2021). While the link between ascospore
207 discharge and leaf spotting is evident (whenever no efficient major gene operating at the leaf level is
208 used), the continuity between earliness/intensity of leaf spotting and severity of stem canker is often
209 questioned, since many factors influence this relationship (duration of cropping, environmental
210 conditions, genotypes used, involvement of other diseases or insect damages, etc.). For example,
211 cultivars susceptible at the leaf spotting stage may be devoid of stem symptoms (Thurling and Venn
212 1977). The reverse may also be found, i.e., the absence of leaf spotting but severe cankers at the end
213 of the growing season (mainly observed in Canada). However, absence of leaf spotting due to the
214 influence of major-effect resistance genes usually results in a low level of stem cankers at the end of
215 the growing season (Bohran et al. 2022).

216 One other important difference in cropping practices to control the disease lies in the use of fungicides,
217 with a constantly increasing use of fungicides at sowing and during the vegetative growth stage in
218 Australia (Van de Wouw et al. 2021), episodic use of fungicides in Western Canada, where the foliar
219 application of fungicides is thought to provide only a limited yield benefit, except when applied to
220 highly susceptible genotypes (Dolatabadian et al. 2022), and no or limited use of fungicides in Europe
221 (Table 1). Thus, the reliance on genetic resistance to control the disease ranges from very high in
222 continental Europe to moderate in Australia, with a somewhat intermediate situation in Canada.

223

224 **Resistance to *L. maculans* in *Brassica* species**

225 *Bases of plant resistance to infection*

226 Plant pathogens and their hosts have undergone a long co-evolution, such that pathogens
227 become adapted to either a few hosts (specialized pathogens) or numerous hosts (generalist
228 pathogens) (Corwin and Kliebenstein 2017). Most interactions between a host and a potential

229 pathogen fail, either because the pathogen is not adapted to infect the host plant or because the plant
230 species has an array of mechanisms to defend itself. The first barrier to be overcome by a potential
231 pathogen is physical/chemical: for example, leaf topography, or the intrinsic presence of chemicals or
232 compounds that pathogens cannot detoxify. Once these barriers are overcome, the pathogen
233 encounters the first immune resistance layer, involving the recognition of pathogen-conserved
234 molecular motifs (such as fungal chitin), and is called Pathogen/Microbe Associated Molecular Pattern
235 (P/MAMP) initiated by plant receptors called Pattern Recognition Receptors (PRR). This recognition
236 triggers basal defenses, known as PAMP Triggered Immunity (PTI), including mechanisms such as
237 stomatal closure to avoid pathogen colonization, and the production of general plant defense
238 compounds. In the course of co-evolution, some pathogen species became able to overcome this
239 recognition via the production of effectors. Effectors are either secondary metabolites, small RNAs, or
240 the well-studied class of small secreted proteins which suppress the host immune system or
241 manipulate its cell physiology to favor penetration and colonization (Lo Presti et al. 2015). The host, in
242 turn, may set up a defense system involving direct or indirect recognition of effectors by “resistance”
243 proteins, encoded by resistance (*R*) genes and activating inducible defenses. Proteinaceous effectors
244 specifically recognized by host resistance proteins are then called Avirulence (*Avr*) proteins. This is
245 referred to as qualitative resistance (depending on a monogenic determinism), as opposed to
246 quantitative resistance (involving polygenic control). The recognition between avirulence and
247 resistance proteins activates the second layer of immunity, called Effector Triggered Immunity (ETI),
248 which results in local cell death: this is generally in the form of a hypersensitive reaction (HR), which
249 prevents the pathogen from spreading further. This interaction is referred to as the gene-for-gene
250 model, in which for a given resistance gene on the host side there is a corresponding avirulence gene
251 on the pathogen side (Flor 1955). Typically, in the literature, most plant *R* genes are said to encode
252 intracellular nucleotide binding-site leucine-rich repeat (NLR, also known as NBS-LRR) proteins, while
253 PAMP-triggered immunity is generally associated with the recognition of M/PAMPs by membrane-
254 associated Receptor-Like Proteins (RLP) or Receptor-Like Kinases (RLK) (Tang et al. 2017). Pathogens
255 can in turn overcome effector-triggered immunity by deletion or mutation of the *Avr* gene. The
256 circumvention of this monogenic resistance is facilitated by biological factors (e.g. population size,
257 reproduction mode) or genomic characteristics (e.g. location of genes encoding avirulence proteins in
258 repeat-rich genomic regions, dispensable chromosomes, subtelomeric regions or large transposable-
259 element (TE) rich regions) (Sánchez-Vallet et al., 2018). In contrast, quantitative resistance is more
260 difficult to circumvent by the pathogen since it relies on several loci (Pilet-Nayel et al. 2015; Niks et al.
261 2015).

262

263 *Overview of the resistance to L. maculans in Brassica species*

264 Historically, at least in Europe where breeding for resistance to *L. maculans* began, plant
265 resistance was evaluated in the field at the end of the long rapeseed cropping season. The plants were
266 thus submitted to a combined load of biotic and abiotic stresses, including mixed avirulent and virulent
267 pathogen populations. Under these conditions, it was virtually impossible to dissect genetic
268 mechanisms underlying resistances, and breeders generally assumed the crop expressed “adult-stage”,
269 multigene, quantitative resistance (e.g., Cargeeg and Thurling 1979). However, some authors
270 postulated very early on that there may be differential behavior of plant genotypes faced with different
271 populations of the fungus, despite still supporting a system of polygenic control/interaction on both
272 the plant and the pathogen side (Thurling and Venn 1977). It was only from the 1990s onwards that
273 studies began to be performed on the fungal side to dissect host specificity, and that rapid miniaturized
274 inoculation tests on plantlets became available, enabling the different components of *Brassica*
275 resistance to *L. maculans* to be dissected. Subsequently, it was demonstrated that qualitative “gene-
276 for-gene” resistance operates in this species as well as quantitative resistance. As many recent reviews
277 have nicely summarized the findings on quantitative resistance of *Brassica* spp. towards *L. maculans*
278 (Amas et al. 2021; Borhan et al. 2022), this paper focuses on critical questions regarding identification,
279 uses, and prospects for durability of qualitative resistances in the *L. maculans* / *Brassica* pathosystem.

280

281 **Methods for identifying qualitative (major effect / gene-for-gene) resistances in the *L. maculans*-*B.*** 282 ***napus* pathosystem**

283 *The cotyledon inoculation test*

284 To bypass the tedious process of field-based assessment of resistance, researchers began to
285 design miniaturized tests to allow screening of a large number of *Brassica* and *L. maculans* genotypes
286 under controlled conditions, in conjunction with the development of reliable scoring methods to assess
287 symptoms. Trials encompassed the use of ascospore suspensions obtained from mixed field residues
288 inoculated on cotyledons without wounding and assessment of cotyledon and stem lesions (Thurling
289 and Venn 1977), single ascospore inoculation of cotyledons (Cargeeg and Thurling 1979), blended
290 mycelium inoculation of cotyledons without wounding (Cargeeg and Thurling 1979), inoculation of the
291 first leaf petiole with paper discs soaked in pycnidiospore suspension after wounding (Newman 1984),
292 inoculation of the lamina of the first true leaf with mixed pycnidiospore suspension after wounding
293 (Mithen et al., 1987) and cotyledon-inoculation test with pycnidiospore suspension from a single
294 isolate after wounding (Mengistu et al. 1991; Badawy et al. 1991). McNabb et al. (1993) compared the
295 accuracy of four different inoculation tests: cotyledon inoculation with cotyledon rating, leaf
296 inoculation with leaf and stem rating, stem inoculation with stem rating, and inoculation using infested
297 oat kernels with stem rating. They concluded that the highest reliability (in terms of reproducibility and

298 positive correlation with field behavior) was attained with the cotyledon test. There have been endless
299 debates on the best inoculation tests to identify relevant R genes, starting from the pioneering work
300 of Mengistu et al. (1991) using a simple inoculation test and a defined rating scale. While a few authors
301 were initially dubious (Newman and Bailey 1987), numerous others subsequently agreed on the high
302 reliability of miniaturized cotyledon tests, which were then rapidly adapted for breeding programs
303 (McNabb et al. 1993).

304 One of the issues with using cotyledon-inoculation tests to identify new sources of resistance
305 is that field isolates of *L. maculans* generally harbor numerous *Avr* genes, rendering it complex to
306 identify if a novel resistance is present in a given genotype. After Mengistu et al. (1991, 1993) identified
307 three *B. napus* genotypes with different pathogen responses and set up protocols for *in vitro* crosses
308 of the fungus, crosses between different fungal isolates under laboratory conditions (Gall et al. 1994)
309 demonstrated that the avirulence phenotypes identified in two *B. napus* genotypes (cvs. 'Quinta' and
310 'Glacier') were under monogenic control in the fungus. This led to the characterization and subsequent
311 cloning of two effector-genes: *AvrLm1* (avirulence against 'Quinta', Ansan-Melayah et al. 1995) and
312 *AvrLm2* (avirulence against 'Glacier'; Ansan-Melayah et al. 1998). These genetic analyses strongly
313 suggested that major resistance genes (*Rlm*) corresponding to avirulence genes existed in rapeseed,
314 which was then validated via genetic analyses on the plant side (Ansan-Melayah et al. 1998).
315 Combining expertise from plant geneticists, plant pathologists, and fungal geneticists, more than 10
316 other avirulence genes were characterized in *L. maculans* and the corresponding resistance genes
317 identified in oilseed rape (*Rlm1*, *Rlm2*, *Rlm3*, *Rlm4*, *Rlm7*, *Rlm9*), as well as in other *Brassica* species
318 such as Indian mustard, *B. juncea* (*Rlm5*, *Rlm6*), black mustard, *B. nigra* (*Rlm10*) and turnip rape, *B.*
319 *rapa* (*Rlm1*, *Rlm3*, *Rlm7*, *Rlm8*, *Rlm11*) (Rouxel and Balesdent 2017). Due to the complexity of *Avr* genes
320 harbored by field isolates, crosses of the fungus were the first option to generate isolates harboring
321 the smallest possible number of *AvrLm* genes and, following a series of backcrosses, near-isogenic
322 isolates differing by only a single *AvrLm* gene (Balesdent et al. 2002; Huang et al. 2010; Rouxel et al.
323 2003b). With the advent of molecular tools, genetic manipulation methods (complementation, RNA
324 silencing of gene expression, and CRISPR-Cas9 gene inactivation), are now routinely used as tools to
325 generate isogenic isolates to identify corresponding *Rlm/LepR* genes in *Brassica* genotypes, for
326 screening genetic resources, or for use in plant breeding (Balesdent et al. 2002; Rouxel et al. 2003b;
327 Ghanbarnia et al. 2012; Van de Wouw et al. 2014; Larkan et al. 2015).

328

329 *Problems with resistance gene identification: nomenclature and availability of genetic resources*

330 Over the years, considerable confusion in resistance gene naming has become evident, with
331 independent naming of genes by independent research groups working on the *L. maculans* / *Brassica*
332 spp. pathosystem. This phenomenon is by no means restricted to this pathosystem, and is also found

333 for instance in the rice–*Magnaporthe oryzae* model (Ballini et al. 2008). This terminology confusion
334 can be attributed to the difficulty in developing and sharing common sets of differential pathogen
335 strains, as well as plant genotypes with an identified and fixed set of resistance genes. In the case of
336 differential isolates in particular, this has been slowed down, or even made fully impossible, by import
337 restrictions established in some parts of the world, which have banned the import of biological
338 material that could be used to demonstrate the identity of a resistance gene in geographically or
339 genetically distant plant material (e.g. in Australia). Considerable difficulty is also involved in tracing
340 and validating the identity of plant genotypes available to the community. Additionally, the initial
341 identification of resistance loci was performed before the *AvrLm-Rlm* gene-for-gene interaction was
342 understood and considered as the rule in this system, and the isolates used for the identification of
343 resistance loci were either field populations or ill-defined isolates in terms of avirulence gene content
344 (Delourme et al. 2006). Some groups even complexified the miniaturized tests by inoculating with
345 mixes of isolates that may be representative of the diversity of their local populations (Gaebelein et al.
346 2019).

347 Nowadays, most of the groups studying the *L. maculans*–*Brassica* interaction use a common
348 *AvrLmX-RlmX/LepRX* terminology. The generation of progeny isolates harboring only a limited and
349 known content of characterized effector/avirulence genes has helped characterized the resistance
350 gene complement of some of the genotypes that were previously employed, and the transposition of
351 markers from one genetic map to another has allowed, with time, previously described resistance
352 genes to be concatenated and assigned (e.g. *LEM1*, *LmFr1*, *LmR1*, *CImR1*, assigned to chromosome A07
353 of *B. napus*, are now assumed to all correspond to *Rlm4* based on map position and phenotypic
354 responses; Rouxel et al. 2003b; Rimmer 2006). The availability of reference genomes and the possibility
355 to physically localize *R* and *AvrLm* genes cleared up a lot of initially confusing results from early linkage
356 mapping studies, where the same gene was often repeatedly detected or where different genes were
357 occasionally conflated (Borhan et al. 2022). Despite these improvements, confusion persists when
358 identifying new resistance or avirulence genes, exemplified by the recent cloning of two avirulence
359 genes in two different research groups, which turned out to be the same gene (i.e. *AvrLmS-LepR2* or
360 *AvrLmJ1/AvrLm5-9*; (Van de Wouw et al. 2014; Plissonneau et al. 2017; Neik et al. 2022).

361 Additional confusion arises when genotypes with the same name harbor different
362 complements of *Rlm* genes, which is sometimes the case when the initial cultivar was not a fixed line,
363 and as such may contain individual plants with different *Rlm* gene complements. Further independent
364 self-pollination events may thus independently fix different *Rlm* genes in different locations (for
365 instance, cv. Tina, with *Rlm3*, *Rlm7*, or no *Rlm* genes present, was used to derive differential lines with
366 *Rlm3* or *Rlm7* (Balesdent et al. 2002); while cv. Quinta could harbor *Rlm1*, *Rlm3* and/or *Rlm4*). This
367 issue of genetic divergence between accessions with the same name may also occur due to the high

368 frequency of unintended outcrossing present in *Brassica* species, an issue long recognized by breeders
369 and researchers. This is an ongoing problem, and although solutions such as a registered accession
370 database have been suggested and may be implemented in time (see e.g. Yang et al. 2023), to date
371 most research groups simply reconfirm resistance gene presence via cotyledon testing with specific
372 isolates after receipt of accessions rather than trusting to published information. However, this
373 additionally emphasizes the importance of coordination in efforts by the international community to
374 identify resistance or avirulence genes.

375

376 *Progress in resistance gene identification*

377 If considering only genes for which a map position has been defined, or for which an avirulence
378 gene has been cloned, at least 18 resistance genes that operate at the cotyledon/leaf stage are known
379 for the *L. maculans*–*Brassica* pathosystem, and one additional gene, *RlmSTEE98*, that operates in the
380 stem (Table 2). Five of these, corresponding to only two distinct loci, have been cloned (Larkan et al.
381 2013, 2015, 2020). Most resistance genes described so far in the A genome of *B. napus* are also present
382 in the A genome of *B. rapa*, such as *Rlm1*, *Rlm2* and *Rlm7* (Leflon et al. 2007). One gene, *Rlm13*, was
383 mapped to chromosome C03 in *B. napus* but is likely a translocation from the homeologous region of
384 A03 (Raman et al. 2021). Few resistance genes have been identified in *B. oleracea* (C genome) with
385 only two described or postulated to date, *Rlm13* and *Rlm14* (Raman et al. 2021; Degraeve et al. 2021).
386 In contrast, a higher number of resistance genes have been identified in diverse *B. rapa* germplasm
387 (*Rlm1*, *Rlm2*, *Rlm7*, *LepR1-4*, *Rlm11*). However, some of these may be identical to those which were
388 previously identified (*RlmS* and *LepR2*, renamed *RlmS-Lep2*; Neik et al. 2022), or may be functional
389 homologs recognizing the same avirulence gene (e.g. *Rlm1* and *LepR3*, which both recognize *AvrLm1*;
390 Larkan et al., 2013; Rouxel and Balesdent 2013). Fewer investigations have been made in other
391 *Brassica* species of less agronomic interest and genetic similarity to *B. napus*, although *Rlm10* has been
392 identified in *B. nigra* (Chevre et al. 1996; Chèvre et al. 1997a, b), and *Rlm5* and *Rlm6* in *B. juncea*
393 (Balesdent et al. 2002; Struss et al. 1991).

394 Several resistances have also been putatively identified but not yet confirmed or mapped as
395 single major genes. It has been suggested that *B. nigra* contains at least two resistance loci on different
396 chromosomes (Rimmer and Van Den Berg 1992; Struss et al. 1996). In cross-progeny between *B.*
397 *carinata* and *B. napus*, the presence of the middle to bottom of chromosome B3 and the top of B8
398 were independently associated with resistance (Fredua-Agyeman et al. 2014). Resistance has also been
399 found in *B. oleracea* var. *capitata* Korean germplasm genotypes (Robin et al. 2017), and putatively in
400 the C genome of the allotetraploid species *B. carinata* (Rahman et al. 2007). In addition, 12 putative R-
401 genes were found in *B. oleracea* within a syntenic region of *LepR4* (Ferdous et al. 2020). While *B. napus*
402 germplasm and, to a lesser extent, *B. oleracea* and *B. rapa* collections of genotypes have been

403 comprehensively investigated to identify sources of resistance, this is much less true of the three
404 mustard species *B. nigra*, *B. juncea*, and *B. carinata*, and it would be logical that this germplasm could
405 also comprise a useful source of diverse new resistance genes, as was the case for *B. rapa*.

406 Table 2: Resistance genes identified in the *Leptosphaeria maculans* – *Brassica* spp. pathosystem

Gene	Species ^a	Genotype ^b	Chromosome ^c	Reference
<i>Rlm1</i>	<i>B. napus</i>	Quinta	A7	Ansan-Melayah et al. 1998
<i>Rlm2*</i>	<i>B. napus</i>	Glacier	A10	Ansan-Melayah et al. 1998; Larkan et al. 2015
<i>Rlm3</i>	<i>B. napus</i>	Glacier, Maxol	A7	Delourme et al. 2004
<i>Rlm4*</i>	<i>B. napus</i>	Jet Neuf	A7	Balesdent et al. 2002; Haddadi et al. 2022
<i>Rlm5</i>	<i>B. juncea</i>	Aurea, Picra	-	Balesdent et al. 2002
<i>Rlm6</i>	<i>B. juncea</i>	Aurea, 150-2-1 and 151-2-1	B8	Chèvre et al. 1997a
<i>Rlm7*</i>	<i>B. napus</i>	Caiman	A7	Balesdent et al. 2002; Haddadi et al. 2022
<i>Rlm8</i>	<i>B. rapa</i>	156-2-1	-	Balesdent et al. 2002
<i>Rlm9*</i>	<i>B. napus</i>	Darmor-bzh	A7	Delourme et al. 2004; Larkan et al. 2020; Haddadi et al. 2022
<i>Rlm10</i>	<i>B. nigra</i>	Junius	B4	Chevre et al. 1996
<i>Rlm11</i>	<i>B. rapa</i>	plant (2323) from line 02-159-4-1	-	Balesdent et al. 2013
<i>Rlm12</i>	<i>B. napus</i>	Skipton/Ag-Spectrum	A1	Raman et al. 2016
<i>Rlm13</i>	<i>B. napus</i>	ATR-Cobbler	C03	Raman et al. 2021
<i>Rlm14</i>	<i>B. oleracea</i>	Monaco	-	Degrave et al. 2021
<i>RlmS-LepR2</i>	<i>B. rapa</i>	Surpass 400	A10	Van De Wouw et al. 2009; Neik et al. 2022
<i>LepR1</i>	<i>B. rapa</i>	DHP95	A2	Yu et al. 2005
<i>LepR3*</i>	<i>B. rapa</i>	Surpass 400	A10	Li and Cowling 2003; Yu et al. 2008 ; Larkan et al. 2013; Larkan et al. 2014; Larkan and Bohran 2015
<i>RlmSTEE98</i>	<i>B. napus</i>	Yudal	A9	Jiquel et al. 2021

407 ^aSpecies in which the gene has been identified; ^bGenotype in which the resistance gene has been first
408 identified; ^cPosition of the resistance gene; *indicates cloned *Rlm* genes; *Rlm4*, *Rlm7*, and *Rlm9* are
409 allelic versions of the same gene; *Rlm2* and *LepR3* are allelic versions of the same gene.

410 *Variability of phenotypic expression in miniaturized inoculation tests*

411 Problems in resistance gene identification may arise from variations in the expression of
412 disease symptoms. These may depend on the genetic background of the *B. napus* accession considered,
413 differences in environmental conditions between locations in which the test is performed, stability of
414 the environmental conditions over time, rating system, and interpretation of the scores. As mentioned
415 previously, although the *Rlm-AvrLm* interaction is under monogenic control, macroscopic expression
416 of the interaction phenotype can still vary. Under controlled conditions, resistance can sometimes
417 result in a localized, complete, and rapid hypersensitive response following inoculation: this is typical
418 of the *AvrLm4-Rlm4* interaction in cultivars 'Jet Neuf' or 'Pixel', or the *AvrLm7-Rlm7* interaction in
419 cultivars 'Caiman' or 'DK Excellium' (Blondeau et al. 2015). Alternatively, the phenotypic expression of
420 resistance can sometimes be diffuse, with more extended necrosis or longer establishment period: this
421 is typical of the *AvrLm1-Rlm1*, *AvrLm3-Rlm3*, and *AvrLm5-Rlm5* interactions (Ansan-Melayah et al. 1995;
422 Van De Wouw et al. 2009). The phenotypic responses can also differ depending on the host genotype.
423 This phenomenon has been regularly reported in the *L. maculans* / *B. napus* pathosystem as well as in
424 other pathosystems (Röhrig and Dussart 2022) and can involve factors such as putative heterozygosity
425 vs. homozygosity, allelic variation, and the host genetic background in which the *R* gene is inserted.
426 The first study to formally address the influence of the host genotype on major gene responses was
427 undertaken in 2019, by Haddadi et al. They introgressed two susceptible genotypes, 'Topas' and
428 'Westar', with the same resistance gene (either *Rlm2*, *Rlm3*, *LepR1*, or *LepR2*) and assessed their
429 phenotypic and transcriptomic behavior when facing an isolate of *L. maculans* having the cognate
430 *AvrLm* genes. Inoculation of 'Topas' and 'Westar' resulted macroscopically in similar large lesions,
431 while inoculation of 'Topas' or 'Westar' in which an *R* gene had been introgressed resulted in different
432 macroscopic expression of resistance. The phenotypic assessment of resistance was mirrored by RNA-
433 seq analysis at the same time points, under infection. This provided an overview of key genes and
434 pathways mobilized in the resistance response, such as genes related to pathways involved in plant
435 defense (salicylic acid, jasmonic acid, abscisic acid, ethylene, and glucosinolate pathways) which are
436 expressed earlier in introgressed-resistance lines compared to susceptible lines. Overall, the authors
437 showed that although similar pathways were upregulated in all introgressed lines post-infection,
438 'Westar'-introgressed lines showed delayed expression of the genes involved in plant defense
439 compared to 'Topas'-introgressed lines. This is the only comprehensive analysis to date of the influence
440 of the host genetic background on phenotypic expression of resistance, and many variable resistance
441 phenotypes remain elusive. This includes the expression of *Rlm7* in the first registered cultivar
442 harboring this resistance, 'Roxet', which is still the only case known in which resistance is not expressed
443 as a typical hypersensitive response. Whether such phenomena may be due to heterozygosity, to less

444 efficient variants of the resistance gene, or an unrelated gene recognizing also *AvrLm4-7* remains to
445 be elucidated.

446 A second source of variation is likely due to the environmental conditions in which the
447 pathogenicity assay is performed. Temperature and wetness influence the phenotypic expression of
448 both major gene and quantitative resistances (Huang et al. 2006, 2009; Larkan et al. 2016; Neik et al.
449 2022). An increase in temperature is associated with a shortening of period before the first symptoms
450 appear on the leaf, and wetness also influenced the number of lesions observed irrespective of
451 genotype (Huang et al. 2009). Recently, the influence of temperature on the expression of the
452 hypersensitive response was also investigated by two different groups (Yang et al. 2021; Noel et al.
453 2022). Yang et al. (2021) tested the expression of the *B. napus* / *L. maculans* interaction under different
454 temperature conditions and showed that lesion size was larger at elevated temperatures and that this
455 was consistently underpinned by differences in the expression of genes associated with plant defense
456 and response to temperature. Noel et al. (2022) also monitored the expression of quantitative
457 resistance in field experiments as well as under controlled conditions, showing that although some
458 cultivars exhibit robust resistance under high temperatures, resistance efficacy may decrease during
459 long heat waves. A better understanding of plant resistance under various environmental conditions
460 is an important field of research to consider in the context of global warming (Röhrig and Dussart 2022).

461 Of major concern is that variation in the resistance response (or its interpretation) may be
462 observed for a given interaction when tests are performed at two different locations with the same
463 control isolate and the same batch of seeds, all freshly shared between two groups, as shown in the
464 study of Neik et al. (2022). One of the hypotheses to explain the variable expression of symptoms and
465 contrasting interpretations of the interaction was a strong influence of environmental conditions on
466 the phenotypic outcome, a hypothesis that remains to be validated. In this specific example, it is
467 interesting to note that despite the divergent interpretation of the phenotypes, the use of crosses
468 involving isolates with contrasting phenotypes on Topas-LepR2 nevertheless allowed for independent
469 cloning of the corresponding avirulence gene *AvrLmS-Lep2*. Use of cultivars like 'Topas', a Canadian
470 line now considered as a universally susceptible genotype (and the source of isogenic lines widely
471 shared in the community; Larkan et al. 2016), but with a general level of resistance higher than the
472 previously used Westar (McNabb et al. 1993), may also result in confusing scores between susceptible
473 and resistant interactions when using European isolates. Whether this is due to an adaptation of
474 Canadian populations to this widely-grown cultivar in Canada and a contrastingly poor adaptation of
475 European isolates remains to be investigated. The complexity of the genetic background and
476 environmental interactions should thus be considered carefully in experiments designed to identify
477 novel sources of resistance.

478

479 **Molecular insights into *AvrLm* and *Rlm* genes**

480 *Genomics methods for candidate resistance gene prediction*

481 Resistance genes can be categorized using their conserved domains and structural features
482 into the three major families of Resistance Gene Analogs (RGA; for review see Sekhwal et al. 2015):
483 nucleotide binding site leucine-rich repeats (NBS-LRR), receptor-like kinases (RLK), and receptor like
484 proteins (RLP). The advent of the genomics era afforded great progress in the elucidation of the
485 genetics underlying the *L. maculans-Brassica* pathosystem (Cantila et al. 2021) together with *in silico*
486 prediction of thousands of RGA in plant genomes. Due to an increase of available plant genomes and
487 pangenomics, attempts have been made to take a “bottom-up” *in silico* approach to *R* gene discovery,
488 working from existing knowledge of expected functional domains in *R* genes (Kruijt et al. 2005) or
489 based on homology to genes previously established to be involved in resistance responses. Using these
490 approaches in *Brassica* spp., *R* genes have been mined from the pangenome of *B. oleracea* and in
491 several other Brassicaceae species, including *Arabidopsis thaliana* (e.g. Bayer et al. 2019; Dolatabadian
492 et al. 2020; Cantila et al. 2022). Resistance gene analog prediction in *B. oleracea* based on pangenome
493 analyses has highlighted that resistance gene content is highly divergent between accessions, and that
494 receptor-like kinases may be the most important class of *Brassica* resistance genes. Transposable
495 elements (TE) and presence/absence gene variation have also been identified as important drivers in
496 generating novel disease resistance and diversification of putative resistance genes (Bayer et al. 2019),
497 showing that a pangenome approach is essential to fully describe and assess the diversity of resistance
498 genes within a species. In *B. napus*, available genomic resources have expanded tremendously in the
499 last decade, from the initial publication of the genome sequence of ‘Darmor-*bzh*’ (Chalhoub et al. 2014)
500 to the first pangenome based on analysis of eight accessions (Song et al. 2020) and the availability of
501 datasets representing genotyping data from c.a. 1,700 accessions (Song et al. 2021) (BnPIR database:
502 <http://cbi.hzau.edu.cn/bnapus/>). This scaling up of available genome data has been mirrored by an
503 extensive effort to predict resistance gene candidates, first in the single sequenced accession ‘Darmor-
504 *bzh*’ with 425 NBS-LRR genes predicted in the first genome assembly (Chalhoub et al. 2014) and more
505 recently based on a *B. napus* pangenome or on available Brassicaceae sequence data (Dolatabadian et
506 al. 2020; Tirnaz et al. 2020). Dolatabadian et al. (2020) predicted 1,749 RGAs across 50 accessions of *B.*
507 *napus*, including almost 400 absent from the reference genome.

508 Despite the huge numbers of *R*-genes predicted through *in silico* analyses in plants and *Brassica* spp.
509 (Bayer et al. 2019; Dolatabadian et al. 2020), individual characterization of these candidates is still a
510 major challenge and limiting factors exist in use of this information for more applied genetics and
511 breeding. Although such studies have provided useful and extensive databases for reference, the
512 primary current use is likely to be in interrogating candidate genes identified to underlie
513 experimentally determined resistance QTL (Cantila et al. 2022), as merely the identification of a

514 resistance gene candidate is not sufficient to know if this gene is in any way involved in any particular
515 pathogen response. However, this method may increasingly gain utility as our understanding of gene
516 and protein interactions, signaling and gene pathways improves, particularly in conjunction with
517 transcriptomic approaches involved in the pathogen infection response (Borhan et al. 2022). A first
518 approach developed to reduce the number of candidates is to compare predicted RGAs with previous
519 knowledge obtained from the genetic mapping of resistance loci to identify robust candidates: such an
520 approach has been carried out already in *B. oleracea* and *B. napus* (Bayer et al. 2019; Dolatabadian et
521 al. 2020). Resistance gene analog prediction can also be compared or applied to transcriptomic data
522 (e.g. Lowe et al. 2014; Becker et al. 2017, 2019; Hubbard et al. 2020; Song et al. 2021) and applied
523 across accessions with known resistance responses and large databases such as BnPIR. This latter
524 approach can be particularly useful, considering the high diversity in terms of gene content between
525 accessions, to assess the conservation of a particular candidate resistance gene analog (Song et al.
526 2020).

527

528 *Validating resistance and avirulence genes*

529 Despite the thousands of resistance gene analogs predicted using bioinformatic pipelines, only a
530 limited number of *R* genes have been cloned, sequenced, and validated in plants (see Kourelis et al.
531 2018 for review). The first *R* gene to be cloned, in 1992, was *Hm1* in *Zea mays*, which encodes an
532 enzyme involved in the detoxification of a toxin produced by the maize pathogen *Cochliobolus*
533 *carbonum* (Johal and Briggs 1992).

534 Within the *B. napus* - *L. maculans* pathosystem, five *Brassica* resistance genes (i.e. *LepR3*, *Rlm2*,
535 *Rlm4*, *Rlm7*, *Rlm9*; Larkan et al. 2013, 2015, 2020; Table 2) and 12 *L. maculans* avirulence genes have
536 been cloned (i.e. *AvrLm1*, *AvrLm2*, *AvrLm3*, *AvrLm4-7*, *AvrLm5-9*, *AvrLm6*, *AvrLm10A*, *AvrLm10B*,
537 *AvrLm11*, *AvrLm14*, *AvrLmS-Lep2*, *LmSTEE98*; Fudal et al. 2007; Gout et al., 2006; Parlange et al. 2009;
538 Balesdent et al. 2013; Ghanbarnia et al. 2015; 2018; Plissonneau et al. 2016; Petit-Houdenot et al. 2019;
539 Degrave et al. 2021; Van de Wouw et al. 2014; Jiquel et al. 2021; Neik et al. 2022; Table 3). This number
540 of avirulence genes cloned is higher than any other crop-disease system so far, comparable to what is
541 known in the *Pyricularia oryzae*-rice model (Hu et al. 2022), making the *Brassica* – *L. maculans*
542 interaction a model system for the study of *R-Avr* gene interactions (reviewed by Rouxel and Balesdent
543 2017; Borhan et al. 2022). Before the availability of reference genomes and high-density genetic maps,
544 identification of candidate genes underlying resistance loci from genetic mapping studies was a major
545 challenge, involving now-obsolete methods (at least in *Brassica*) such as chromosome walking
546 (reviewed by Jander et al. 2002). The major methods still used for candidate gene identification and
547 functional validation are often classified as “forward genetics” and “reverse genetics” methods.
548 Forward genetics firstly involves inspection and functional annotation of candidate genes underlying

549 mapped trait loci to predict which genes are most likely causal for the trait of interest: this can be
550 facilitated by identifying the “type” of genes present and their predicted function (annotation).
551 Subsequently, sequencing a subset of susceptible and resistant lines can determine if the two groups
552 share different, putatively functional allelic variants of the same candidate gene, which is then likely
553 to be directly related to the resistance phenotype. Reverse genetic approaches involve going from
554 genotype to phenotype, and include mutant screening methods such as TILLING (McCallum et al. 2000)
555 where gene mutants are first identified before the phenotypic effect of the mutation is experimentally
556 verified: reverse genetics approaches have to date not been used in the *Brassica-L. maculans*
557 pathosystem, but may have potential in the future for validating candidate genes identified through
558 genomic prediction methods.

559

560 *Cloning of avirulence genes*

561 Forward genetic approaches have been very successful in cloning avirulence genes in the *L. maculans*
562 / *B. napus* pathosystem, with *AvrLm1*, the first *AvrLm* gene cloned in 2006 (Gout et al. 2006). This gene
563 was previously genetically identified by Ansan-Melayah et al. (1995) using a map-based cloning
564 strategy which allowed the identification of the genetic interval on a BAC (Bacterial Artificial
565 Chromosome) contig. This cloning highlighted interesting characteristics of *AvrLm1* and its genomic
566 environment: located in the middle of a large TE-rich region, *AvrLm1* itself encodes a small protein
567 (205 amino-acids) with one cysteine residue and a peptide signal and has no homology with other
568 sequences available in public databases. It was later shown that these characteristics (except for
569 cysteine enrichment) were shared by all avirulence genes that were subsequently cloned
570 (subsequently referred to as SSPs for Small Secreted Proteins), together with low or no expression
571 during axenic growth and specific induction during the asymptomatic infection of *B. napus*, at the
572 beginning of the infection period (except for *LmSTEE98*; Rouxel et al. 2011; Jiquel et al. 2021). *AvrLm1*,
573 *AvrLm6*, and *AvrLm4-7* were all cloned using the same strategy (Gout et al. 2006; Fudal et al. 2007;
574 Parlange et al. 2009) before the release of the *L. maculans* genome (Rouxel et al. 2011). Since then,
575 availability of the genomic sequence of *L. maculans* has facilitated cloning of additional avirulence
576 genes: an exception was *AvrLm3* which required a combination of several strategies for successful
577 cloning (map-based cloning, RNA-seq analysis, BAC sequencing; Plissonneau et al. 2016), most likely
578 because it is located in a telomeric region, surrounded by repeats and absent from the initial reference
579 genome assembly of *L. maculans*. Cloning of *AvrLmS-Lep2* was undertaken using two independent
580 approaches: a typical MAP-based cloning approach, and bulked-segregant sequencing of progeny
581 produced by crossing between virulent and avirulent isolates (Neik et al. 2022). Avirulence genes
582 *AvrLep2* and *AvrLmS* were previously shown to trigger resistance in cultivar ‘Surpass400’ of *B. napus*

583 which contains *RlmS* and *LepR2*. Cloning showed that these are the same gene, which was therefore
584 named *AvrLmS-Lep2*.

585 All avirulence genes cloned to date are initially overexpressed following the infection of oilseed rape,
586 where interaction between an *AvrLm* gene and its cognate *Rlm* gene triggers a resistance response at
587 the onset of the infection. Nevertheless, the recent characterization of the *AvrLmSTEE98/RlmSTEE98*
588 interaction highlighted that a gene-for-gene interaction could be involved in the limitation of stem
589 colonization, and triggering of partial resistance phenotypes (Jiquel et al. 2021). Examples such as
590 these will undoubtedly help rethink our current categories of “qualitative” and “quantitative”
591 resistances in the *L. maculans* / *Brassica* pathosystem and open the way to identification of further
592 gene-for-gene interactions expressed at other plant growth stages, and/or on other plant organs than
593 previous gene-for-gene interactions uncovered so far in this pathosystem at the cotyledon/leaf stage.

594

595 *Cloning of resistance genes*

596 Although the first *AvrLm* gene was cloned in 2006, it was only in 2013 that the first major resistance
597 gene in *B. napus* against stem canker was cloned (*LepR3*; Larkan et al. 2013). Cloning and characterizing
598 *Rlm* and *Avrlm* genes generally follows a standard approach: firstly, identification of genotypes with
599 opposite phenotypic characteristics (susceptible and resistant lines, for the plant species; avirulent and
600 virulent isolates for the fungus), followed by one, or several, cross(es) between these genotypes, then
601 genotypic and phenotypic analysis of resulting segregating progeny to identify genomic regions
602 associated with the resistance phenotype. Distribution of the resistance/virulence phenotype across
603 phenotypic categories as well as Mendelian segregation ratios can help in determining if one or more
604 genes/loci are responsible for the trait. Depending on the size of the resulting associated region/s,
605 candidate genes may then also be investigated within these regions, usually firstly by functional
606 annotation (gene ontology predictions based on sequence comparisons to genes of known function),
607 then by validation of gene function. The most common method of functional validation involves the
608 transformation (cloning and insertion) of the candidate resistance gene into a susceptible background
609 and screening of the transformants for resistance.

610

611 *LepR3* was mapped on chromosome A10 in 2008 (Yu et al. 2008), then cloning from the cultivar
612 ‘Surpass400’ (Li et al. 2004, 2007) was finally achieved in 2013. To achieve cloning of *LepR3*, the authors
613 developed an elegant approach to identify candidate genes, with the improvement of the genetic map
614 to provide a high-resolution mapping of *LepR3* coupled with the use of data from closely-related
615 species *B. rapa* and *A. thaliana* to investigate collinearity between species for the *LepR3* region (Larkan
616 et al. 2013). The *B. rapa* region containing the *LepR3* genetic interval was then annotated, and
617 subsequent sequencing of the corresponding locus from *B. napus* allowed for the identification of a

618 candidate gene encoding a Receptor-Like Protein (RLP). The parental genotypes both had the genetic
619 locus, but the resistant cultivar ‘Surpass400’ had a longer coding sequence for the gene than the
620 susceptible cultivar ‘Topas DH16516’, as well as sequence variations. *LepR3* triggers a hypersensitive
621 response during interaction with *AvrLm1*-isolates of *L. maculans*, which is also involved in a gene-for-
622 gene interaction with *Rlm1* (Larkan et al. 2013; Table 3). Since the cloning of *LepR3* by Larkan and
623 colleagues, the same authors successfully cloned four other *Rlm* genes (*Rlm2*, *Rlm4*, *Rlm7*, and *Rlm9*;
624 Larkan et al. 2015, 2020; Haddadi et al. 2022). The second *Rlm* gene to be cloned was *Rlm2* (Larkan et
625 al. 2015), inducing a hypersensitive response during interaction with *AvrLm2*-isolates of *L. maculans*
626 (Table 3). It was previously shown that *Rlm2* and *LepR3* were located in the same genetic interval
627 (Larkan et al. 2014) and amplification of the candidate gene before transformation of a susceptible
628 genotype for gene functional validation purposes was performed using the same primers as for
629 amplification of *LepR3*. The authors have shown that *LepR3* and *Rlm2* are alleles of the same gene, yet
630 trigger a hypersensitive response during interaction with isolates containing *AvrLm1* or *AvrLm2*,
631 respectively. Besides these two RLP-encoding genes, *Rlm9*, the third cloned *Rlm* gene, is a wall-
632 associated kinase-like (WAKL) encoding gene (Larkan et al. 2020). The genetic interval was previously
633 identified on chromosome A07 of the cultivar ‘Darmor-bzh’ (Larkan et al. 2016; Table 2) and forms a
634 genetic cluster with *Rlm4*, *Rlm3*, and *Rlm7*. Haddadi et al. (2022) recently showed that *Rlm4* and *Rlm7*
635 are alleles of *Rlm9*, and it is postulated that *Rlm3* is yet another allele of the same gene. Cloning of *Rlm*
636 genes demonstrated that different *Rlm* genes can correspond to alleles of the same gene, or might
637 correspond to the same gene and that different resistance genes can trigger resistance via interaction
638 with the same *AvrLm* gene (for instance, both *LepR3* and *Rlm1* trigger resistance toward *AvrLm1*; Table
639 3). Larkan et al. (2013) and Rouxel and Balesdent (2013) postulated that *Rlm1* and *LepR3* could be the
640 same gene, although they occupy different genomic loci, with *Rlm1* on chromosome A07 and *LepR3*
641 on chromosome A10. A translocation of *Rlm1* could have given rise to *LepR3* in ‘Surpass400’ (Van De
642 Wouw et al. 2009), or ‘Surpass400’ could contain *Rlm1*, *LepR3*, and *RlmS*. Despite the cloning of *AvrLm*
643 and *Rlm* genes, much still needs to be elucidated given the complexity of the pathosystem, and more
644 and more complex situations have been uncovered as the number of cloned genes has increased.

645 In contrast to many other pathosystems in which NLR-intracellular receptors are the rule, cloned *Rlm*
646 genes so far mostly correspond to RLP-encoding genes. So far, *Rlm9* and *Stb6* (a resistance gene
647 operating in wheat against *Zymoseptoria tritici*) are the only WAKL resistance genes to be cloned
648 (Saintenac et al. 2018). This indicates that RLP or RLK types are not only associated with PAMP-
649 triggered immunity, but may also be main players in effector-triggered immunity against apoplastic
650 pathogens such as *Z. tritici*, *L. maculans*, or *Fulvia fulvum*.

651

652 *Expression of Rlm and effector (including AvrLm) genes*

653 Cloning of *AvrLm* and *Rlm* genes together with the development of more and more advanced
654 gene expression analysis techniques has allowed a deeper understanding of their expression during
655 the whole infection cycle, including spatial and temporal regulation. At first, analysis of *AvrLm* gene
656 expression was done upon cloning using qRT-PCR, then a broader analysis of gene expression was
657 made possible using microarray technology following the publication of the genome sequence of *L.*
658 *maculans*, and most recently, RNA-seq analysis has allowed an in-depth exploration of the
659 transcriptome (Gay et al. 2021; unpublished data). Accumulation of these data and the increase in data
660 quality has deepened our understanding of the complexities of effector-gene expression. RT-PCR
661 analysis of *AvrLm1* expression showed an upregulation of this gene 7 to 12 days-post infection (Gout
662 et al. 2006). Microarray analysis highlighted coordinated expression of a set of effector genes, including
663 all cloned *AvrLm* genes together with other effector candidates, with low (or no) expression during
664 axenic growth and a strong over-expression during the asymptomatic infection of *B. napus*. Specifically,
665 *AvrLm* genes and other effector candidate genes showed a peak of expression seven days post
666 inoculation, before the appearance of the first visible symptoms on cotyledons (Rouxel et al. 2011). A
667 better knowledge of effector-gene expression was rendered possible with the first RNA-seq analyses
668 performed at different stages of the interaction between *L. maculans* and *B. napus* (Gervais et al. 2017).
669 The authors performed RNA-seq analysis on cotyledons and stem, showing that a subset of candidate
670 effector-genes were expressed specifically during stem colonization and suggesting a possible role of
671 these effectors in necrosis establishment at this later stage of the lifecycle. These were renamed 'late'
672 effector-genes to distinguish them from *AvrLm* genes specifically induced during primary
673 asymptomatic infection of *B. napus*. Analysis of a later infection stage and a different plant organ than
674 cotyledons (from which most transcriptomic data were generated so far) revealed a more complex
675 expression of effector-genes and suggested different roles of subsets of effectors that are expressed
676 at distinct life stages. Gene expression analysis throughout the life cycle of *L. maculans* (Gay et al. 2021)
677 revealed a very complex regulation of the genes involved in pathogenesis, far more sophisticated than
678 initially postulated from analyses carried out during early infection of cotyledons or petioles under
679 controlled conditions (Rouxel et al. 2011; Gervais et al. 2017; Gay et al. 2021). Based on previous
680 analyses of *AvrLm* gene expression from microarray data (Rouxel et al. 2011) or from the first RNA-seq
681 analysis performed on stems (Gervais et al. 2017), the situation remained quite simple, with a subset
682 of effectors, the *AvrLm* genes, expressed solely during primary infection. This suggested the
683 involvement of these genes in manipulating the host immune defense system to ensure establishment
684 of the infection. The thorough analysis of the *L. maculans* transcriptome throughout the life cycle,
685 under controlled conditions, and in the field, in different plant organs (e.g. cotyledons, leaf, stem,
686 residues), covering different life stages of the fungus (e.g. mycelium, ascospores) and all lifestyles
687 displayed by the fungus (asymptomatic, necrotrophic, endophytic, saprophytic) compared to axenic

688 culture (on several media) has deepened our understanding of the complexities of (candidate)
689 effector-gene expression. RNA-seq data showed that eight specific clusters of genes, all enriched in
690 effector genes, were expressed during interaction with oilseed rape and specifically associated with a
691 given lifestyle and/or an infected tissue. Gay et al. (2021) showed that expression of *AvrLm* genes was
692 finely regulated such that activation occurs exclusively during the different asymptomatic colonization
693 stages, not only during primary infection of cotyledons or leaves.

694 In contrast to effector genes, much is yet to be discovered regarding *Rlm* gene expression.
695 Becker et al. (2017) performed RNA-seq and gene expression analysis using qRT-PCR following laser
696 microdissection to provide a thorough analysis of the global response of a resistant and a susceptible
697 genotype of *B. napus* together with a specific analysis of the infection site. Differentially expressed
698 genes underlying the infection response in resistant genotypes were enriched with genes involved in
699 pathogen recognition, cell signaling, and vesicular trafficking, and included genes encoding NBS-LRR
700 receptors, wall-associated kinases, RLK,s and RLPs (Becker et al. 2017). The recent cloning of *Rlm* genes
701 also made it possible to investigate their expression during infection. For instance, *Rlm9* was
702 specifically up-regulated in 'Darmor' (containing *Rlm9*) compared to a susceptible genotype following
703 infection with an *AvrLm5-9* isolate (Larkan et al. 2020). Moreover, *Rlm9* shows a temporal expression
704 pattern, with a low induction at three days post-inoculation, a peak of expression at six days post-
705 inoculation, and a slight decrease in expression up to nine days post-inoculation. This pattern
706 resembles that of the *AvrLm* genes cloned so far.

Table 3: Avirulence genes in *L. maculans* and known *R* gene interactions with *Brassica* species

<i>L. maculans</i> <i>AvrLm</i> genes	Cognate <i>Rlm</i> genes	Notes	<i>AvrLm</i> gene cloned	Reference
<i>AvrLm1</i>	<i>Rlm1</i> <i>LepR3</i> *	-	Yes	Gout et al. 2006
<i>AvrLm2</i>	<i>Rlm2</i> *	<i>Rlm2</i> and <i>LepR3</i> are alleles of the same gene	Yes	Ghanbarnia et al. 2015
<i>AvrLm3</i>	<i>Rlm3</i>	Presence of <i>AvrLm4-7</i> masks interaction of <i>Rlm3</i> and <i>AvrLm3</i>	Yes	Plissonneau et al. 2016
<i>AvrLm4-7</i>	<i>Rlm4</i> <i>Rlm7</i>	<i>Rlm4</i> , <i>Rlm7</i> , and <i>Rlm9</i> are different allelic versions of the same gene	Yes	Parlange et al. 2009 Ghanbarnia et al. 2018
<i>AvrLm5-9</i> ^a	<i>Rlm5</i> <i>Rlm9</i> *	<i>Rlm5</i> and <i>Rlm9</i> are alleles of the same gene; presence of <i>AvrLm4-7</i> masks interaction of <i>Rlm9</i> and <i>AvrLm5-9</i>	Yes	Ghanbarnia et al. 2018; Van De Wouw et al. 2014; Plissonneau et al. 2018
<i>AvrLm6</i>	<i>Rlm6</i>	-	Yes	Fudal et al. 2009
<i>AvrLm8</i>	<i>Rlm8</i>	-	No	Balesdent et al. 2002
<i>AvrLm10A</i>	<i>Rlm10</i>	<i>AvrLm10A</i> and <i>AvrLm10B</i> are both required to trigger resistance	Yes	Petit-Houdenot et al. 2019
<i>AvrLm10B</i>	<i>Rlm10</i>		Yes	
<i>AvrLm11</i>	<i>Rlm11</i>	-	Yes	Balesdent et al. 2013
<i>AvrLm13</i> ^b	<i>Rlm13</i>	-	No	Raman et al. 2021
<i>AvrLm14</i>	<i>Rlm14</i>	-	Yes	Degrave et al. 2021
<i>AvrLmS-Lep2</i>	<i>RlmS</i> <i>LepR2</i>	-	Yes	Neik et al. 2022
<i>AvrLepR1</i> ^c	<i>LepR1</i>	-	No	Ghanbarnia et al. 2012; Yu et al. 2005
<i>AvrLepR2</i>	<i>LepR2</i>	-	No	Yu et al. 2005
<i>AvrLepR4</i> ^b	<i>LepR4</i>	-	No	Yu et al. 2013
<i>AvrLmSTEE98</i> ^d	<i>RlmSTEE98</i>	-	Yes	Jiquel et al. 2021

707 ^a*AvrLm5-9* was first named *AvrLmJ1* (van de Wouw et al. 2014) and then identified as *AvrLm5* by
708 Plissonneau et al. (2018). Finally, Ghanbarnia et al. (2018) identified the double specificity towards
709 *Rlm5* and *Rlm9*,

710 ^bThis avirulence gene is only postulated to date since it has not been genetically identified or mapped,

711 ^cThis gene has been genetically identified and mapped, but not cloned,

712 ^d*AvrLmSTEE98* encodes for an avirulence protein recognized during stem colonization.

713 *: indicates cloned *Rlm* genes

714 *Complex interactions between R genes and AvrLm genes*

715 To date, *L. maculans* – *Brassica* spp. is the pathosystem for which the largest number of
716 avirulence genes have been cloned so far, along with several of the corresponding plant resistance
717 genes. This has highlighted that interactions between avirulence proteins and their cognate R proteins
718 do not always follow the classic gene-for-gene interaction model, with more and more peculiar cases
719 uncovered as new avirulence genes are cloned (such as two-for-one models, different allelic
720 interactions, and epistatic suppression of phenotypes).

721 As previously mentioned, an important characteristic of the gene-for-gene model is the discovery of
722 increasing complexity, invalidating the assumption that one gene is always related to another single
723 gene. Several discoveries of such complex interactions have been recorded, such as *AvrLm1*, which is
724 recognized by *LepR3* and *Rlm1* (Larkan et al. 2013; Table 3). Another example is the two-gene for one
725 gene interaction between both *AvrLm10A* and *AvrLm10B* and *Rlm10*. These two avirulence genes are
726 necessary to induce avirulence towards *Rlm10* (Petit-Houdenot et al. 2019; Talbi et al. 2023). Some
727 other avirulence genes have dual recognition specificities, such as *AvrLm4*, which was renamed
728 *AvrLm4-7* as it can generate a resistance response in the presence of both *Rlm4* and *Rlm7* (Parlange et
729 al. 2009), or *AvrLm5-9* that induces responses to both *Rlm5* and *Rlm9* (Van De Wouw et al. 2014;
730 Ghanbarnia et al. 2018;). The fourth series of examples illustrate the “camouflage model” whereby
731 one avirulence may mask the recognition of another avirulence by the matching resistance. There are
732 two examples here. Firstly, *AvrLm4-7* hides the presence of the *AvrLm3* isolate and prevents
733 recognition by *Rlm3*, even when *AvrLm3* is present and expressed (Plissonneau et al. 2016). Deletions
734 or inactivating mutations of *AvrLm4-7* lead to unmasking and recognition of *AvrLm3*, while other
735 mutations such as those generating virulent isoforms of the *AvrLm3* protein, or isolates that contain
736 point mutations in *AvrLm4-7*, escape *Rlm7* resistance while maintaining the suppression of the *AvrLm3*
737 phenotype (Plissonneau et al. 2017; Balesdent et al. 2022). These studies show that the *AvrLm3* gene,
738 once thought to be lost due to the high selection pressure caused by widespread *Rlm3*-containing
739 cultivars, is still present and expressed (Rouxel and Balesdent 2017). A second example is the *AvrLm5-9*
740 host recognition, which is also masked by the presence of *AvrLm4-7*. The presence of *AvrLm4-7* masks
741 the recognition of *AvrLm5-9* by *Rlm9*, similarly to what is known for the *AvrLm4-7* masking of *AvrLm3*.
742 *AvrLm5-9* and *AvrLm4-7* do not interact, and the presence of *AvrLm4-7* does not suppress the
743 expression of *AvrLm5-9*.

744

745 **A history of resistance gene use and the evolution of populations on different continents**

746 *Use of resistance genes in Europe, Canada and Australia*

747 As mentioned previously, breeding for resistance in *B. napus* was initially based on mass
748 selection, picking up the more resistant plants at the end of the growing season for further breeding,
749 under the assumption that the resistance was under quantitative genetic control (Cowling 2007). These
750 approaches inadvertently identified and selected major gene resistances, which were the most
751 efficient genetic factors for control of the disease in a context where the dominant race of the fungus
752 was locally avirulent. The increasing popularity of inbreeding to generate pure, homogenous lines also
753 contributed to the impoverishment of R gene diversity present in the initial varieties (see the example
754 of ‘Tina’ or ‘Quinta’ above), eventually generating cultivars harboring a single resistance gene. In many
755 cases retrospective analysis using genetically bred isolates allowed identification of the resistance
756 genes used in early breeding strategies, whenever the genotypes were still available in collections.

757 Europe, being the first to breed for improved genotypes with high agronomic value, and being
758 faced early with the stem canker problem, was also the first to breed (unknowingly) for major *Rlm*
759 genes (Rouxel and Balesdent 2017). In France, for example, *Rlm2* was released in commercial cultivars
760 in 1970, *Rlm4* in 1971 (with extreme commercial success at the European scale when introduced in cv.
761 ‘Jet Neuf’ in 1977), *Rlm1* in 1992 (with extreme commercial success when introduced in cv. ‘Capitol’ in
762 1995), and *Rlm7* in 2002 (with an extreme commercial success when introduced in cultivars such as
763 ‘Exocet’ and ‘Exagone’ from 2004 on) (for review see Rouxel and Balesdent 2017). In addition, *Rlm3*
764 and *Rlm9* have been present for years in cultivars, but there is no definite information on when and in
765 which cultivars they were first released (e.g. for *Rlm3* in Balesdent et al. 2022), and without displaying
766 any efficient resistance towards populations of the fungus that were 100% virulent at the European
767 scale (Stachowiak et al. 2006). More recently, two *syvestris*-derived genes were introduced in winter
768 type European cultivars: *RlmS-Lep2* in 2016 (Balesdent et al. 2023), and *LepR1* in 2021 (M.-H. Balesdent
769 and A. Jiquel, unpublished data). It has also to be mentioned that *Rlm1*, *Rlm2*, *Rlm3*, *Rlm4*, and *Rlm9*
770 have been (or are still) maintained in cultivars long after they have been defeated (Balesdent et al.
771 2022; M.-H. Balesdent, unpublished data).

772 In Australia, the first resistance gene used was *Rlm4*, in 1974 (Roy and Reeves, 1975; Rouxel et
773 al. 2003b) followed in 2000 by the use of the *syvestris*-derived resistance (*RlmS-Lep2* and *LepR3* –
774 firstly identified as *Rlm1*) by Van De Wouw et al. (2009). From the late 1990s onwards, Australian
775 canola breeders began to incorporate European winter germplasm, especially from France, more
776 extensively into their breeding programs, allowing diversification of the *Rlm* gene complement
777 (Marcroft et al. 2012). In an extensive screening of 127 Australian cultivars and advanced breeding
778 lines done in the early 2010s, 29% contained *Rlm4*, 27% contained *Rlm1* (or *LepR3*), 21% contained
779 *Rlm9*, 10% contained *RlmS-Lep2*, 9% contained *Rlm3*, and 5% contained *Rlm2* (Marcroft et al. 2012).
780 More recent data suggest *Rlm6*, introgressed from *B. juncea*, is also available in some Australian

781 cultivars (Van de Wouw and Howlett 2020). However, the use of specific resistance genes in Australia
782 is sometimes unclear due to variable naming conventions and changes in gene content in the same
783 genotype from one paper to the other. Mainly, the *sylvestris*-derived resistance genes in ‘Surpass400’
784 were first identified as *Rlm1* and *RlmS* (Van De Wouw et al. 2009), with confusion between *LepR3* and
785 *Rlm1* (both recognizing *AvrLm1*), while *RlmS* was later identified as *LepR2* and renamed *RlmS-Lep2*
786 (Neik et al. 2022). However, in subsequent papers (van de Wouw et al. 2016; 2018), ‘Surpass400’ was
787 described to harbor *Rlm1* and *LepR3*, and *LepR3* was confused with *RlmS-Lep2*. Thus, *RlmS-Lep2* was
788 either *LepR3* or more recently *LepR1*, while the *LepR1* gene identified in Canada and recently released
789 in Europe is different from *RlmS-Lep2* (M.-H. Balesdent and A. Jiquel, unpublished data). Also, *Rlm5*
790 (from *B. juncea*) is suggested to be absent from Australian genotypes but corresponds to one resistance
791 group in the Australian classification of resistant cultivars (Van de Wouw et al. 2017; Van de Wouw
792 and Howlett 2020). In Australia, there are strong incentives to rotate resistance genes as much as
793 possible to alternate selection pressures on the pathogen, and only local breakdowns of resistance
794 have been documented, likely due to the lack of cropping of one single resistance gene on large
795 acreages for extended periods, in contrast to what is observed in Europe.

796 In Canada, the first documented R gene used was *Rlm3*, released in 1995. *Rlm3* was then used
797 in numerous varieties for years, and was present in up to 55% of the registered varieties in the mid-
798 2010s (Zhang et al. 2016). While *LepR3/Rlm1*, *Rlm2*, *Rlm3*, *Rlm4*, *Rlm9*, *RlmS-Lep2*, and *LepR1* have
799 been documented to be present in Canadian genotypes, at present, only four R genes are commercially
800 available in Canadian varieties: *LepR3/Rlm1*, *Rlm3*, *Rlm4* and *RlmS-Lep2* (Zhang et al. 2016; Cornelsen
801 et al. 2021).

802

803 *The durability of resistance in the L. maculans / B. napus pathosystem*

804 The wide use of major resistance genes in crops is famed for rapidly selecting populations of
805 the pathogen that have become virulent. The speed of this “breakdown” is theoretically linked to the
806 “evolutionary potential” of the pathogen (McDonald and Linde 2002), but also depends on more
807 complex traits including the fitness cost for the pathogen to lose the *AvrLm* protein effector function,
808 the complexity of the gene-for-gene interaction, and the pre-existence of virulent isolates in the
809 populations. *L. maculans* sums up a series of biological and epidemiological traits that allows it to
810 “breakdown”, in a few years only, novel resistance genes deployed over large areas due to their
811 commercial success. These include a mixed reproduction regime and an obligate annual sexual
812 reproduction favoring recombination of avirulence loci, very high local population size (ascospores
813 resulting from sexual reproduction and produced for most of the vegetative life of rapeseed), and
814 location of avirulence genes in plastic regions of the genome which undergo accelerated mutation

815 rates. Thus, the generation of novel virulent isolates which can break down novel resistance genes is
816 mostly a local and self-sustaining process, and virulence generated at each sexual cycle is selected and
817 amplified in populations whenever a new resistance is used (Rouxel and Balesdent 2017).

818 Historically, in Europe, *Rlm* gene use has led to the breakdown of resistance at the country
819 scale for *Rlm2* (in 1972 in France), *Rlm1* (in 1999 in France), *Rlm4* (possibly at the end of the 1990s),
820 and *Rlm7* (in 2022 in France) (Rouxel and Balesdent 2017; Balesdent et al. 2022). The absence of
821 virulent isolates at the *AvrLm2* and *AvrLm9* loci in Europe may also indicate an ancient, undocumented
822 breakdown of these two resistance genes. Another well-documented breakdown event is that of the
823 *sylvestris* resistance (*LepR3* and *RlmS-Lep2*), present in ‘Surpass400’, in the Eyre Peninsula (Australia)
824 in 2003 and then in other parts of Australia (Sprague et al. 2006). Finally, Western Canada experienced
825 an *Rlm3* breakdown between 1997 and 2010 (Zhang and Fernando, 2018). The speed of these different
826 breakdown events differed between the different situations, and this evaluation may depend on the
827 definition given by the authors of “breakdown”: in other words, when do we consider that a resistance
828 gene has “broken down”? This may depend on the geographic scale considered, the ratio of virulent
829 to avirulent isolates in the population, the amount of yield losses, or other factors. Thus, the literature
830 documents very rapid breakdowns, e.g., the 5-year breakdown of *Rlm1* at the French scale (with more
831 than 90% of the isolates in populations having shifted to *AvrLm1* virulence) or of the 3-year breakdown
832 of *LepR3* (also favoring the evolution of isolates virulent at the *AvrLm1* locus) in the Eyre Peninsula
833 (Australia) (Rouxel et al. 2003a; Sprague et al. 2006). It is also probable that the breakdown of *Rlm2* in
834 France has been a very rapid event with only two years between the release of a successful *Rlm2*
835 cultivar and documented susceptibility of the cultivar (Rouxel and Balesdent 2017). However, for
836 reasons which are still mostly elusive, and may differ between regions, plant genotypes, cropping
837 practices, and complex interactions between *AvrLm* genes and resistance genes themselves, such rapid
838 breakdowns are not always the rule. For example, the breakdown of *Rlm3* in Canada was more a slow
839 erosion of the efficiency of the gene over decades than a rapid breakdown, even though *Rlm3* was
840 widely used in Canadian varieties (Zhang and Fernando 2018). Similarly, and contrasting with what has
841 been observed in experimental settings (Daverdin et al. 2012), the breakdown of *Rlm7* in France,
842 massively deployed since 2004, has been a rather slow process (Balesdent et al. 2022). All of this
843 indicates that a priori postulation on the durability of an *Rlm* gene is elusive, and necessitates
844 knowledge of the importance of the *AvrLm* gene for the fungus, its mutability, and the existence of
845 epistatic effects masking other avirulence genes (Balesdent et al. 2022).

846 The durability of the resistance must also consider the possible pre-existence of virulent
847 isolates in populations. While it is now well-established in Europe that virulent isolates for *AvrLm1*,
848 *AvrLm7*, or *AvrLmS-Lep2* loci were extremely rare at the time of introduction of the corresponding

849 resistance gene (Rouxel et al. 2003a; Balesdent et al. 2022, 2023), this may not be the case in Australia
850 or Canada, reinforcing the ease with which new resistances are broken down. For example, a survey
851 done in Western Canada by Dimaghani et al. (2009) in 2004-2006 indicated that virulence at *AvrLm1*,
852 *AvrLm4*, *AvrLm7*, or *AvrLm9* loci predated the use of the resistance in commercial varieties and that
853 extreme diversity exists even between different locations in the same province. In Manitoba (Canada),
854 some locations have 100% of isolates virulent at the *AvrLm1* locus, while others have up to 62%
855 avirulent isolates. Similar disparities were found for *AvrLm4* or *AvrLm7*, with some locations harboring
856 100% virulent isolates, while others have up to 75% avirulent isolates (Dilmaghani et al. 2009). Similar
857 findings were obtained by Cornelsen et al. (2021) with a high number of isolates virulent at the *AvrLmS-*
858 *Lep2* locus, suggesting virulent isolates were widely present in the population before the use of the
859 corresponding *Rlm* gene in commercial varieties. While such extensive surveys have not been
860 performed in Australia, a retrospective examination of a collection of 26 isolates (including two from
861 New Zealand) collected between 1971 and 2002 by Balesdent et al. (2005) indicated that 77% of the
862 isolates were virulent towards *Rlm4*, consistent with the use of this resistance gene in Australian
863 varieties, but also that 54% were virulent at the *AvrLm1* locus and 65% at the *AvrLm7* locus. This pre-
864 existence of virulent isolates may have aided the rapid breakdown of the 'Surpass400' resistance, as
865 already postulated by Sprague et al. (2006), and, more recently, aided the breakdown of *Rlm7* in two
866 fields in Victoria (Van de Wouw et al. 2022). These data point to another major difference between
867 Europe and Australia/Canada: while in Europe, successive use of *Rlm* genes had a strong effect on
868 fungal population structure, eliminating one by one avirulence at loci *AvrLm1*, *AvrLm2*, *AvrLm4*,
869 *AvrLm7* and *AvrLm9* from the populations, the situation is more complex for Australia and Western
870 Canada, with a high diversity of races, often at the local scale, independent of the use of *Rlm* genes,
871 and with virulent races predating the use of the corresponding *Rlm* genes.

872 *Population surveys to aid durable management of resistance*

873 To maximize the durability of cultivars with new resistance, precise information on race structure is
874 needed. This could be achieved by scouting the races present in the field at different scales prior to
875 and during the use of these cultivars, leading to the development of strategies to maximize the
876 durability of a rare resource. To avoid the use of tedious and space-consuming pathogenicity tests,
877 molecular markers for each *AvrLm* gene are tools of choice to speed up population characterization
878 and improve screening throughput. The design of such markers requires that the molecular events
879 leading to virulence are extensively characterized and are followed up on to track down the dynamics
880 of events that lead to a fixed virulent allele (Rouxel and Balesdent 2017; Gautier et al. 2023).
881 Unfortunately, there may be only limited information for some of the genes at present in the literature,
882 and over-simplification of available data may lead to inaccurate recommendations for the best markers

883 to use. In general, when the fungal populations are challenged by a new resistance, a common
884 immediate response is the elimination of the *AvrLm* gene via drastic events (Gout et al. 2007; Daverdin
885 et al. 2012; Fudal et al. 2009). This is facilitated by the hosting of these genes in large TE-rich regions
886 of the genome, and obligate reproduction favoring repeat-induced point mutation (RIP) events at each
887 sexual cycle. As a consequence, the first step to virulence is either complete deletion or mutation to
888 inactivation via RIP (Rouxel and Balesdent 2017). In France, this was observed for *AvrLm1* and *AvrLm7*
889 in agricultural settings (Rouxel et al. 2013a; Balesdent et al. 2022) and for *AvrLm6* and *AvrLm7* in
890 experimental fields (Fudal et al. 2009; Daverdin et al. 2012). However, even when hosted in TE-rich
891 regions, including subtelomeric regions, some genes (such as *AvrLm2*, *AvrLm3*, or *AvrLm9*) are never
892 deleted or inactivated, and a virulent allele has formed by point mutation (or mutations) and replaced
893 the avirulent form in populations (except when remaining masked via epistatic interactions as found
894 for *AvrLm3*; see below). In contrast, another gene, *AvrLm11*, is spontaneously lost due to being located
895 in a dispensable chromosome of *L. maculans*, even in the absence of selection pressure (Balesdent et
896 al. 2013). For currently used resistance genes in Europe, three cases can thus be distinguished. The
897 first is where *AvrLm* genes corresponding to *Rlm* genes have been present in genotypes for ages and
898 have led to fixed alleles, probably following a first round of drastic events such as deletions. There are
899 different cases here: genes that are deleted and never recovered (*AvrLm1* being the sole example
900 known) and genes in which the avirulent allele has been replaced by a virulent one, resulting in a single
901 amino acid substitution (as in *AvrLm2*, *AvrLm4*, and *AvrLm9*). The second case is where *AvrLm* genes
902 correspond to *Rlm* genes that have never been used in commercial varieties, and for which there is
903 only limited information on molecular evolution toward virulence in agricultural settings. Thirdly,
904 *AvrLm* genes may also be masked from recognition by the matching *Rlm* gene due to epistatic
905 interactions between *AvrLm* genes. In this respect, a recent comprehensive study has been performed
906 by Gautier et al. (2023) that identified numerous alleles at each avirulence locus, and showed that the
907 diversity of alleles found in a population is gene-dependent and independent of the selection pressure
908 to which the population has been submitted. This work is currently complemented by a large
909 international effort to gather and sequence numerous *L. maculans* isolates from all parts of the world
910 and to capture their diversity at avirulence loci (van de Wouw et al. submitted). These genomic factors
911 driving rapid diversification at *AvrLm* loci need to be considered when deploying new resistance genes,
912 and extensive data on sequence variation at avirulence/virulence loci will be instrumental to designing
913 molecular markers to survey populations of the pathogen.

914

915 **Sustainable management: breeding strategies to build genotypes with durable resistance**

916 Breeding for resistant cultivars of *B. napus* is widely agreed to be one of the best management
917 strategies for ensuring crop production while under pressure from the *L. maculans* pathogen (reviewed
918 by Borhan et al. 2022). However, ensuring the durability of the genetic resistance in the crop cultivars
919 can be challenging. The fungal pathogen is to date able to break down all resistances known in *B. napus*,
920 and genetic resources which would enable further diversification of R genes within commercial
921 cultivars are tapped out within the primary *B. napus* germplasm pool. From this perspective,
922 sustainable use and rotation of existing resistances within the crop (Van de Wouw et al. 2021) coupled
923 with pathogen-informed management strategies (reviewed by Li et al. 2020) are critical strategies for
924 sustaining yield in *Brassica napus*. To effectively match resistance in the cultivars with existing isolate
925 populations, a great deal of information is required: the exact R gene composition of the cultivars
926 involved, the *Avr* gene content of the pathogen populations at the cropping location, and any possible
927 effects of gene interactions, environmental effects, and cultivar genetic backgrounds on the R-*Avr*
928 interaction in the field. Despite the complexity, this is still an achievable strategy, particularly with
929 recent advances in high-throughput genotyping and our understanding of this crop-pathogen
930 interaction (Borhan et al. 2022).

931

932 *A critical discussion of the pyramiding strategy*

933 A major strategy which may help build durable resistances into cultivars is that of gene pyramiding
934 (Stam and McDonald 2018). In this strategy, cultivars are developed to contain multiple resistance
935 genes, allowing for broad-spectrum resistance against a range of isolate populations. In a broader
936 sense, pyramiding may be the combination of major gene resistance with quantitative resistance
937 within a single cultivar. This specific strategy is likely to offer the best protection against both major
938 gene breakdown and the possibility that the fungus will evolve under pressure to simultaneously
939 overcome multiple major resistance genes (Leflon et al. 2007; Pilet-Nayel et al. 2017). In all cases,
940 pyramiding of exclusively major resistance genes in a single genotype has to be carefully thought over,
941 keeping in mind possible adverse effects and the possibility of facilitating resistance breakdown across
942 multiple genes. This was illustrated by the combination of *Rlm3 – Rlm7* in current French varieties that
943 favored a rapid adaptation in the fungus to generate double-virulent isolates with a single mutation in
944 the avirulence gene (Balesdent et al. 2022), an outcome that should be avoided at all costs. This points
945 again to the importance of preliminary dissection of the types of interactions that may exist between
946 avirulence genes.

947 Combining major gene resistance and quantitative resistance may also be insufficient. In Australia from
948 1970 to 2000 for instance, breeding for polygenic blackleg resistance was possible thanks to diverse
949 ancestral parents, but by 2000 there were signs of loss of genetic diversity and genetic drift leading to

950 the breakdown of the quantitative resistance background (Cowling 2007). Combining quantitative and
951 major gene / qualitative resistance sources may however still offer a viable breeding strategy for the
952 future, particularly when coupled with a better understanding of quantitative resistances and the
953 development of molecular markers which can help to accurately detect QTL and integrate them into
954 breeding programs (Pilet-Nayel et al. 2017). In particular, the recent finding that some adult-stage
955 resistances may be under simple genetic control (Jiquel et al. 2021, 2022) may favor the generation of
956 markers to easily construct genotypes harboring both major gene resistance (operating at the leaf level)
957 combined with a series of single gene quantitative resistances (operating during stem colonization).

958 *Resistance genes in related species: an opportunity to be seized*

959 One of the greatest challenges for the future will be the diversification of the available R gene
960 pool. This is a critical point to design strategies to ensure durable use of the few resistance sources
961 available, and to avoid sequential marketing of one gene after the other in a way that facilitates
962 breakdown and has been the rule in Europe since the early 1970s. To achieve this goal in inbred *B.*
963 *napus*, it will be necessary to look to related species (Katche et al. 2019). *Brassica napus* germplasm
964 can be readily enriched by using resynthesis: genotypes of progenitor species *B. rapa* × *B. oleracea* can
965 be utilized to directly produce 2n = AACC types, which are optimal for further breeding (Katche et al.
966 2023). Direct crossing between *B. rapa* and *B. napus* can also be carried out, followed by selection for
967 euploid (2n = AACC) progeny; likewise crosses between *B. oleracea* and *B. napus*, although these often
968 require embryo rescue (reviewed by FitzJohn et al. 2007). All crosses involving non-adapted germplasm
969 can however be challenging for breeding programs: wild relatives generally carry undesirable
970 agronomic traits, which complicates the use of this material for breeding purposes (Sevis et al. 2003).
971 However, one successful example of the use of resynthesized *B. napus* is the cultivar ‘Varola 50 syn
972 Surpass 400’ (Anon 2001), which contains many resistance genes coming from the A genome of *B. rapa*
973 (Table 1).

974 The *Brassica* B genome is a particularly good source of stem canker resistance genes for
975 breeding purposes (Navabi et al. 2013), but one which is difficult to use for rapeseed crop improvement.
976 Resistance genes found in *Brassica* A or C genomes of related species can be transferred directly into
977 *B. napus* in interspecific hybrids via homologous recombination, but B genome resistance genes rely
978 on the much rarer recombination events between divergent genomes (homoeologous – ancestrally
979 homologous) to transfer resistance genes (Chèvre et al. 2018). Previous attempts to introgress B
980 chromosomes from *B. carinata* into *B. napus* to transfer stem canker disease resistance were not
981 successful, but B-genome chromosomes were inherited as whole linkage groups and some were
982 retained in further generations (Navabi et al. 2011). Dhaliwal et al. (2017) identified B-genome
983 introgressions from *B. carinata* into *B. napus*, but stem canker disease resistance was not assessed in

984 this study (the trait of interest was pod-shatter resistance). Dixelius and Wahlberg (1999) studied the
985 conservation of specific regions of the B genome from *B. nigra*, *B. juncea*, and *B. carinata* in a BC₃
986 population backcrossed to *B. napus*, but found no B-genome chromosomes or chromosome arms.
987 However, a few successful introgressions of resistance into *B. napus* have been recorded, such as the
988 case of the introgression of the *Rlm10* gene on chromosome B4 of *B. nigra* (Chevre et al. 1996) or the
989 transfer of *Rlm6* from *B. juncea* to *B. napus* (Balesdent et al. 2002).

990

991 *Structural families of AvrLm/effector-genes*

992 In addition to these somewhat “classical” approaches to increase the number of available
993 resistances, new prospects may arise through molecular engineering of selected plant immune
994 receptors. This strategy is based on the new finding that fungal effectors, despite showing little or no
995 sequence similarity, may belong to structural families (Outram et al. 2022). Structural families may be
996 found in the same fungal species (like the MAX effectors in *Magnaporthe*, or the LARS family in *L.*
997 *maculans*; Lazar et al. 2022), and thus may be recognized by closely related resistance proteins, that
998 may ideally be engineered to increase their recognition specificities (Cesari et al. 2022). Interestingly,
999 structural families may also be common to many fungal genera, providing the potential to engineer
1000 multispecies recognition and resistance. This is for example the case for the LARS effector family of *L.*
1001 *maculans* that encompasses *AvrLm3*, *AvrLm4-7*, and *AvrLm5-9*, but also the *Ecp11-1* effector of the
1002 unrelated fungal species *F. fulvum* (Lazar et al. 2022). The recent release and improvements of the
1003 deep learning neural network-based prediction software AlphaFold (Jumper et al. 2021) have already
1004 allowed structural prediction for thousands of fungal effectors (Seong and Krasileva 2023), heralding a
1005 new era in understanding effector recognition and making steps towards *in silico* engineering of
1006 resistance genes (Outram et al. 2022).

1007

1008 **Conclusions**

1009 Breeding for stem canker disease resistance is important, as it is one of the major diseases of
1010 rapeseed/canola worldwide. Major strategies currently in place to improve stem canker resistance in
1011 rapeseed are 1) identification and exploitation of genes present in different commercial *B. napus*
1012 cultivars for crop rotation and management purposes; 2) identification and utilization of quantitative
1013 genetic resistance, preferably also via pyramiding with existing major gene loci; and 3) identification
1014 of novel sources of stem canker resistance in diverse germplasm and wild relatives, and subsequent
1015 transfer to the cultivated crop gene pools. In the future, the development of molecular engineering

1016 approaches based on structural predictions of resistance proteins and further advances in
1017 bioinformatics and biotechnology may also substantially contribute to the production of resistant lines.
1018 A combination of these approaches may allow us to develop sustainable blackleg stem canker
1019 management strategies for rapeseed crop production.

1020

1021 **References**

1022 Amas J, Anderson R, Edwards D, et al (2021) Status and advances in mining for blackleg (*Leptosphaeria*
1023 *maculans*) quantitative resistance (QR) in oilseed rape (*Brassica napus*). Theor Appl Genet.
1024 <https://doi.org/10.1007/s00122-021-03877-0>

1025 Anon. (2001) Descriptions *Brassica napus* var. oleifera Canola.'Varola 50' syn 'Surpass 400'. Plant
1026 Varieties J. 14:36-37

1027 Ansan-Melayah D, Balesdent M-H, Buee M, Rouxel T (1995) Genetic characterization of *AvrLm1*, the
1028 first avirulence gene of *Leptosphaeria maculans*. Phytopathology 85:1525–1529.
1029 <https://doi.org/10.1094/phyto-85-1525>

1030 Ansan-Melayah D, Balesdent M-H, Delourme R, et al (1998) Genes for race-specific resistance against
1031 blackleg disease in *Brassica napus* L. Plant Breed 117:373–378. <https://doi.org/10.1111/j.1439-0523.1998.tb01956.x>

1033 Badawy HMA, Hoppe H-H, Koch E (1991) Differential reactions between the genus *Brassica* and
1034 aggressive single spore isolates of *Leptosphaeria maculans*. J Phytopathol 131:109–119.
1035 <https://doi.org/10.1111/j.1439-0434.1991.tb04737.x>

1036 Balesdent M-H, Gautier A, Plissonneau C, et al (2022) Twenty years of *Leptosphaeria maculans*
1037 population survey in France suggests pyramiding *Rlm3* and *Rlm7* in rapeseed is a risky resistance
1038 management strategy. <https://doi.org/10.1094/PHYTO-04-22-0108-R>.
1039 <https://doi.org/10.1094/PHYTO-04-22-0108-R>

1040 Balesdent M-H, Laval V, Bagot P, Rouxel T (2023) Large-scale population survey of *Leptosphaeria*
1041 *maculans* in France highlights both on-going breakdowns and potentially effective resistance
1042 genes in oilseed rape. *Pest Manag Sci* <https://doi.org/10.1002/ps.7401>

1043 Balesdent M-H, Attard A, Kühn ML, Rouxel T (2002) New avirulence genes in the phytopathogenic
1044 fungus *Leptosphaeria maculans*. Phytopathology 92:1122–1133.
1045 <https://doi.org/10.1094/phyto.2002.92.10.1122>

1046 Balesdent M-H, Barbeti MJ, Li H, et al (2005) Analysis of *Leptosphaeria maculans* race structure in a
1047 worldwide collection of isolates. Phytopathology 95:1061–1071.
1048 <https://doi.org/10.1094/PHYTO-95-1061>

1049 Balesdent M-H, Fudal I, Ollivier B, et al (2013) The dispensable chromosome of *Leptosphaeria maculans*

1050 shelters an effector gene conferring avirulence towards *Brassica rapa*. *New Phytol* 198:887–898.
1051 <https://doi.org/10.1111/nph.12178>

1052 Ballini E, More JB, Droc G, et al (2008) A genome-wide meta-analysis of rice blast resistance genes and
1053 quantitative trait loci provides new insights into partial and complete resistance. *Mol Plant-*
1054 *Microbe Interact* 21:859–868. <https://doi.org/10.1094/MPMI-21-7-0859>

1055 Bayer PE, Golicz AA, Tirnaz S, et al (2019) Variation in abundance of predicted resistance genes in the
1056 *Brassica oleracea* pangenome. *Plant Biotechnol J* 17:789–800.
1057 <https://doi.org/10.1111/pbi.13015>

1058 Becker MG, Haddadi P, Wan J, et al (2019) Transcriptome analysis of *Rlm2*-mediated host immunity in
1059 the *Brassica napus*–*Leptosphaeria maculans* pathosystem. *Mol Plant-Microbe Interact* 32:1001–
1060 1012. <https://doi.org/10.1094/MPMI-01-19-0028-R>

1061 Becker MG, Zhang X, Walker PL, et al (2017) Transcriptome analysis of the *Brassica napus*–
1062 *Leptosphaeria maculans* pathosystem identifies receptor, signaling and structural genes
1063 underlying plant resistance. *Plant J* 90:573–586. <https://doi.org/10.1111/tpj.13514>

1064 Bell JM (2012) From rapeseed to canola: a brief history of research for superior meal and edible oil.
1065 *Poult Sci* 61:613–622. <https://doi.org/10.3382/ps.0610613>

1066 Blondeau K, Blaise F, Graille M, et al (2015) Crystal structure of the effector *AvrLm4-7* of *Leptosphaeria*
1067 *maculans* reveals insights into its translocation into plant cells and recognition by resistance
1068 proteins. *Plant J* 83:610–624. <https://doi.org/10.1111/tpj.12913>

1069 Bonman JM, Gabrielson RL, Williams PH, Delwiche P. (1981) Virulence of *Phoma lingam* to cabbage.
1070 *Plant Dis* 65:865. <https://doi.org/10.1094/pd-65-865>

1071 Borhan MH, Van De Wouw AP, Larkan NJ (2022) Molecular interactions between *Leptosphaeria*
1072 *maculans* and *Brassica* species. *Annu Rev Phytopathol* 60:237–257.
1073 <https://doi.org/10.1146/annurev-phyto-021621-120602>

1074 Brun H, Chèvre AM, Fitt BD, et al (2010) Quantitative resistance increases the durability of qualitative
1075 resistance to *Leptosphaeria maculans* in *Brassica napus*. *New Phytol* 185:285–299.
1076 <https://doi.org/10.1111/j.1469-8137.2009.03049.x>

1077 Cai X, Huang Y, Jiang D, et al (2018) Evaluation of oilseed rape seed yield losses caused by *Leptosphaeria*
1078 *biglobosa* in central China. *Eur J Plant Pathol* 150:179–190. <https://doi.org/10.1007/s10658-017-1266-x>

1080 Canola Council of Canada (2021) Canola encyclopedia: Blackleg.
1081 <https://www.canolacouncil.org/canola-encyclopedia/diseases/blackleg/>. Accessed 4 Jan 2023

1082 Cantila AY, Neik TX, Tirnaz S, et al (2022) Mining of cloned disease resistance gene homologs (CDRHs)
1083 in *Brassica* species and *Arabidopsis thaliana*. *Biology (Basel)* 11:821.
1084 <https://doi.org/10.3390/biology11060821>

1085 Cantila AY, Saad NSM, Amas JC, et al (2021) Recent findings unravel genes and genetic factors
1086 underlying *Leptosphaeria maculans* resistance in *Brassica napus* and its relatives. Int J Mol Sci
1087 22:1–25. <https://doi.org/10.3390/ijms22010313>

1088 Cargeeg LA, Thurling N (1979) Seedling and adult plant resistance to blackleg (*Leptosphaeria maculans*
1089 (Desm.) ces. et de not.) in spring rape (*Brassica napus* L.). Aust J Agric Res 31:37–46.
1090 <https://doi.org/10.1071/AR9800037>

1091 Cesari S, Xi Y, Declerck N, et al (2022) New recognition specificity in a plant immune receptor by
1092 molecular engineering of its integrated domain. Nat Commun 13:1–13.
1093 <https://doi.org/10.1038/s41467-022-29196-6>

1094 Chalhoub B, Denoeud F, Liu S, et al (2014) Early allopolyploid evolution in the post-neolithic *Brassica*
1095 *napus* oilseed genome. Science 345:950–953. <https://doi.org/10.1126/science.1253435>

1096 Chèvre A-M, Mason AS, Coriton O, et al (2018) Cytogenetics, a Science Linking Genomics and Breeding:
1097 The *Brassica* Model. Springer, Cham, pp 21–39

1098 Chèvre AM, Barret P, Eber F, et al (1997a) Selection of stable *Brassica napus*-*B. juncea* recombinant
1099 lines resistant to blackleg (*Leptosphaeria maculans*). 1. Identification of molecular markers,
1100 chromosomal and genomic origin of the introgression. Theor Appl Genet 95:1104–1111.
1101 <https://doi.org/10.1007/s001220050669>

1102 Chèvre AM, Eber F, Barret P, et al (1997b) Identification of the different *Brassica nigra* chromosomes
1103 from both sets of *B. oleracea*-*B. nigra* and *B. napus*-*B. nigra* addition lines with a special emphasis
1104 on chromosome transmission and self-incompatibility. Theor Appl Genet 94:603–611.
1105 <https://doi.org/10.1007/s001220050457>

1106 Chevre AM, Eber F, This P, et al (1996) Characterization of *Brassica nigra* chromosomes and of blackleg
1107 resistance in *B. napus*-*B. nigra* addition lines. Plant Breed 115:113–118. <https://doi.org/DOI>
1108 [10.1111/j.1439-0523.1996.tb00884.x](https://doi.org/10.1111/j.1439-0523.1996.tb00884.x)

1109 Cornelsen J, Zou Z, Huang S, et al (2021) Validating the strategic deployment of blackleg resistance
1110 gene groups in commercial canola fields on the Canadian prairies. Front Plant Sci 12:902.
1111 <https://doi.org/10.3389/fpls.2021.669997>

1112 Corwin JA, Kliebenstein DJ (2017) Quantitative Resistance: More Than Just Perception of a Pathogen.
1113 Plant Cell 29:655–665. <https://doi.org/10.1105/tpc.16.00915>

1114 Cowling WA (2007) Genetic diversity in Australian canola and implications for crop breeding for
1115 changing future environments. Field Crop Res 104:103–111.
1116 <https://doi.org/10.1016/j.fcr.2006.12.014>

1117 Darpoux H, Louvet J, Ponchet J (1957) Essais de traitement des semences de crucifères contre le *Phoma*
1118 *lingam* (Tode) Desm. et l'*Alternaria brassicae* (Berk.) Sacc. Ann Epiphyties 57:545–557

1119 Daverdin G, Rouxel T, Gout L, et al (2012) Genome structure and reproductive behaviour influence the

1120 evolutionary potential of a fungal phytopathogen. PLoS Pathog 8: e1003020.
1121 <https://doi.org/10.1371/journal.ppat.1003020>

1122 Degrave A, Wagner M, George P, et al (2021) A new avirulence gene of *Leptosphaeria maculans* ,
1123 *AvrLm14* , identifies a resistance source in American broccoli (*Brassica oleracea*) genotypes. Mol
1124 Plant Pathol 00:1–14. <https://doi.org/10.1111/mpp.13131>

1125 Delourme R, Chèvre AM, Brun H, et al (2006) Major gene and polygenic resistance to *Leptosphaeria*
1126 *maculans* in oilseed rape (*Brassica napus*). Eur J Plant Pathol 114:41–52.
1127 <https://doi.org/10.1007/s10658-005-2108-9>

1128 Delourme R, Pilet-Nayel ML, Archipiano M, et al (2004) A cluster of major specific resistance genes to
1129 *Leptosphaeria maculans* in *Brassica napus*. Phytopathology 94:578–583.
1130 <https://doi.org/10.1094/phyto.2004.94.6.578>

1131 Dhaliwal I, Mason AS, Banga S, et al (2017) Cytogenetic and molecular characterization of B-Genome
1132 introgression lines of *Brassica napus* L. G3 Genes, Genomes, Genet 7:77–86.
1133 <https://doi.org/10.1534/g3.116.036442>

1134 Dilmaghani A, Balesdent M-H, Didier JP, et al (2009) The *Leptosphaeria maculans* - *Leptosphaeria*
1135 *biglobosa* species complex in the American continent. Plant Pathol 58:1044–1058.
1136 <https://doi.org/10.1111/j.1365-3059.2009.02149.x>

1137 Dilmaghani A, Gladieux P, Gout L, et al (2012) Migration patterns and changes in population biology
1138 associated with the worldwide spread of the oilseed rape pathogen *Leptosphaeria maculans*. Mol
1139 Ecol 21:2519–2533. <https://doi.org/10.1111/j.1365-294X.2012.05535.x>

1140 Dixelius C, Wahlberg S (1999) Resistance to *Leptosphaeria maculans* is conserved in a specific region
1141 of the *Brassica* B genome. Theor Appl Genet 99:368–372.
1142 <https://doi.org/10.1007/s001220051246>

1143 Dixon GR (2006) Vegetable brassicas and related crucifers. CABI.
1144 <https://10.1079/9780851993959.0000>

1145 Dolatabadian A, Bayer PE, Tirnaz S, et al (2020) Characterization of disease resistance genes in the
1146 *Brassica napus* pangenome reveals significant structural variation. Plant Biotechnol J 18:969–982.
1147 <https://doi.org/10.1111/pbi.13262>

1148 Dolatabadian A, Cornelsen J, Huang S, et al (2022) Sustainability on the farm: breeding for resistance
1149 and management of major canola diseases in Canada contributing towards an IPM approach. Can
1150 J Plant Pathol 44:157–190. <https://doi.org/10.1080/07060661.2021.1991480>

1151 FAO (2023) FAOSTAT. <http://www.fao.org/faostat/en/#data/QC/visualize>. Accessed 6 Mar 2023

1152 Ferdous MJ, Hossain MR, Park JI, et al (2020) In-silico identification and differential expressions of
1153 *LepR4*-syntenic disease resistance related domain containing genes against blackleg causal
1154 fungus *Leptosphaeria maculans* in *Brassica oleracea*. Gene Reports 19:100598.

1155 <https://doi.org/10.1016/j.genrep.2020.100598>

1156 Fitt BDL, Brun H, Barbetti MJ, Rimmer SR (2006) World-wide importance of phoma stem canker
1157 (*Leptosphaeria maculans* and *L. biglobosa*) on oilseed Rape (*Brassica napus*). Eur J Plant Pathol
1158 114:3–15. <https://doi.org/10.1007/s10658-005-2233-5>

1159 FitzJohn RG, Armstrong TT, Newstrom-Lloyd LE, et al (2007) Hybridisation within *Brassica* and allied
1160 genera: Evaluation of potential for transgene escape. Euphytica 158:209–230.
1161 <https://doi.org/10.1007/s10681-007-9444-0>

1162 Flor HH (1955) Host-parasite interactions in flax rust - its genetic and other implications.
1163 Phytopathology 45:680–685

1164 Fredua-Agyeman R, Coriton O, Huteau V, et al (2014) Molecular cytogenetic identification of B genome
1165 chromosomes linked to blackleg disease resistance in *Brassica napus* × *B. carinata* interspecific
1166 hybrids. Theor Appl Genet 127:1305–1318. <https://doi.org/10.1007/s00122-014-2298-7>

1167 Friedt W, Tu J, Fu T (2018) Academic and economic importance of *Brassica napus* rapeseed. Springer,
1168 Cham, pp 1–20. https://doi.org/10.1007/978-3-319-43694-4_1

1169 Fudal I, Ross S, Brun H, et al (2009) Repeat-Induced Point Mutation (RIP) as an alternative mechanism
1170 of evolution toward virulence in *Leptosphaeria maculans*. Mol Plant Microbe 22:932–941.
1171 <https://doi.org/10.1094/mpmi>

1172 Fudal I, Ross S, Gout L, et al (2007) Heterochromatin-like regions as ecological niches for avirulence
1173 genes in the *Leptosphaeria maculans* genome: Map-based cloning of *AvrLm6*. Mol Plant-Microbe
1174 Interact 20:459–470. <https://doi.org/10.1094/MPMI-20-4-0459>

1175 Gaebelein R, Alnajar D, Koopmann B, Mason AS (2019) Hybrids between *Brassica napus* and *B. nigra*
1176 show frequent pairing between the B and A/C genomes and resistance to blackleg. Chrom Res
1177 27:221–236. <https://doi.org/10.1007/s10577-019-09612-2>

1178 Gall C, Balesdent M-H, Robin P, Rouxel T (1994) Tetrad analysis of acid phosphatase, soluble protein
1179 patterns, and mating type in *Leptosphaeria maculans*. Phytopathology 84:1299–1305.
1180 <https://doi.org/10.1094/Phyto-84-1299>

1181 Gautier A, Balesdent, M-H, Faure S, Laval V, Rouxel T (2023) Polymorphism of avirulence alleles genes
1182 and adaptation to *Brassica* resistance genes is gene-dependent in the phytopathogenic fungus
1183 *Leptosphaeria maculans*. Phytopathology <https://doi.org/10.1094/PHYTO-12-22-0466-R>

1184 Gay EJ, Soyer JL, Lapalu N, et al (2021) Large-scale transcriptomics to dissect 2 years of the life of a
1185 fungal phytopathogen interacting with its host plant. BMC Biol 19:1–27.
1186 <https://doi.org/10.1186/s12915-021-00989-3>

1187 Gervais J, Plissonneau C, Linglin J, et al (2017) Different waves of effector genes with contrasted
1188 genomic location are expressed by *Leptosphaeria maculans* during cotyledon and stem
1189 colonization of oilseed rape. Mol Plant Pathol 18:1113–1126.

1190 <https://doi.org/10.1111/mpp.12464>

1191 Ghanbarnia K, Fudal I, Larkan NJ, et al (2015) Rapid identification of the *Leptosphaeria maculans*
1192 avirulence gene *AvrLm2* using an intraspecific comparative genomics approach. *Mol Plant Pathol*
1193 16:699–709. <https://doi.org/10.1111/mpp.12228>

1194 Ghanbarnia K, Lydiate DJ, Rimmer SR, et al (2012) Genetic mapping of the *Leptosphaeria maculans*
1195 avirulence gene corresponding to the *LepR1* resistance gene of *Brassica napus*. *Theor Appl Genet*
1196 124:505–513. <https://doi.org/10.1007/s00122-011-1724-3>

1197 Ghanbarnia K, Ma L, Larkan NJ, et al (2018) *Leptosphaeria maculans* *AvrLm9*: a new player in the game
1198 of hide and seek with *AvrLm4-7*. *Mol Plant Pathol* 19:1754–1764.
1199 <https://doi.org/10.1111/mpp.12658>

1200 Gout L, Fudal I, Kuhn ML, et al (2006) Lost in the middle of nowhere: The *AvrLm1* avirulence gene of
1201 the Dothideomycete *Leptosphaeria maculans*. *Mol Microbiol* 60:67–80.
1202 <https://doi.org/10.1111/j.1365-2958.2006.05076.x>

1203 Gout L, Kuhn ML, Vincenot L, et al (2007) Genome structure impacts molecular evolution at the *AvrLm1*
1204 avirulence locus of the plant pathogen *Leptosphaeria maculans*. *Environ Microbiol* 9:2978–2992.
1205 <https://doi.org/10.1111/j.1462-2920.2007.01408.x>

1206 Gugel RK, Petrie GA (1992) History, occurrence, impact, and control of blackleg of rapeseed. *Can J Plant*
1207 *Pathol* 14:36–45. <https://doi.org/10.1080/07060669209500904>

1208 Gupta SK, Pratap A (2007) History, Origin, and Evolution. *Adv Bot Res* 45:1–20.
1209 [https://doi.org/10.1016/S0065-2296\(07\)45001-7](https://doi.org/10.1016/S0065-2296(07)45001-7)

1210 Haddadi P, Larkan NJ, Borhan MH (2019) Dissecting R gene and host genetic background effect on the
1211 *Brassica napus* defense response to *Leptosphaeria maculans*. *Sci Rep* 9:1–13.
1212 <https://doi.org/10.1038/s41598-019-43419-9>

1213 Haddadi P, Larkan NJ, Van deWouw A, et al (2022) *Brassica napus* genes *Rlm4* and *Rlm7*, conferring
1214 resistance to *Leptosphaeria maculans*, are alleles of the *Rlm9* wall-associated kinase-like
1215 resistance locus. *Plant Biotechnol J* 20:1229–1231. <https://doi.org/10.1111/pbi.13818>

1216 Hammond KE, Lewis BG, Musa TM (1985) A systemic pathway in the infection of oilseed rape plants by
1217 *Leptosphaeria maculans*. *Plant Pathol* 34:557–565. <https://doi.org/10.1111/J.1365-3059.1985.TB01407.X>

1218

1219 Henderson MP (1918) The black-leg disease of cabbage caused by *Phoma lingam*. *Phytopathology*
1220 8:379–431

1221 Hu ZJ, Huang YY, Lin XY, et al (2022) Loss and natural variations of blast fungal avirulence genes
1222 breakdown rice resistance genes in the Sichuan basin of China. *Front Plant Sci* 13:907.
1223 <https://doi.org/10.3389/fpls.2022.788876>

1224 Huang YJ, Balesdent M-H, Li ZQ, et al (2010) Fitness cost of virulence differs between the *AvrLm1* and

1225 *AvrLm4* loci in *Leptosphaeria maculans* (phoma stem canker of oilseed rape). *Eur J Plant Pathol*
1226 126:279–291. <https://doi.org/10.1007/s10658-009-9539-7>

1227 Huang YJ, Evans N, Li ZQ, et al (2006) Temperature and leaf wetness duration affect phenotypic
1228 expression of *Rlm6*-mediated resistance to *Leptosphaeria maculans* in *Brassica napus*. *New*
1229 *Phytol* 170:129–141. <https://doi.org/10.1111/j.1469-8137.2006.01651.x>

1230 Huang YJ, Mitrousis GK, Sidique SNM, et al (2018) Combining R gene and quantitative resistance
1231 increases effectiveness of cultivar resistance against *Leptosphaeria maculans* in *Brassica napus*
1232 in different environments. *PLoS One* 13:e0197752.
1233 <https://doi.org/10.1371/journal.pone.0197752>

1234 Huang YJ, Pirie EJ, Evans N, et al (2009) Quantitative resistance to symptomless growth of
1235 *Leptosphaeria maculans* (phoma stem canker) in *Brassica napus* (oilseed rape). *Plant Pathol*
1236 58:314–323. <https://doi.org/10.1111/j.1365-3059.2008.01957.x>

1237 Huang YJ, Qi A, King GJ, Fitt BDL (2014) Assessing Quantitative resistance against *Leptosphaeria*
1238 *maculans* (phoma stem canker) in *Brassica napus* (oilseed rape) in young plants. *PLoS One*
1239 9:e84924. <https://doi.org/10.1371/journal.pone.0084924>

1240 Hubbard M, Zhai C, Peng G (2020) Exploring mechanisms of quantitative resistance to *Leptosphaeria*
1241 *maculans* (Blackleg) in the cotyledons of canola (*Brassica napus*) based on transcriptomic and
1242 microscopic analyses. *Plants* 9:864. <https://doi.org/10.3390/plants9070864>

1243 Jacques N, Balesdent M-H, Rouxel T, Laval V (2020) New specific quantitative real-time PCR assays shed
1244 light on the epidemiology of two species of the *Leptosphaeria maculans*–*Leptosphaeria biglobosa*
1245 species complex. *Plant Pathol* 70:643–654. <https://doi.org/10.1111/PPA.13323>

1246 Jander G, Norris SR, Rounsley SD, et al (2002) *Arabidopsis* map-based cloning in the post-genome era.
1247 *Plant Physiol* 129:440–450. <https://doi.org/10.1104/pp.003533>

1248 Jiquel A, Gay EJ, Mas J, et al (2022) “Late” effectors from *Leptosphaeria maculans* as tools for
1249 identifying novel sources of resistance in *Brassica napus*. *Plant Direct* 6:e435.
1250 <https://doi.org/10.1002/pld3.435>

1251 Jiquel A, Gervais J, Geistodt-Kiener A, et al (2021) A gene-for-gene interaction involving a ‘late’ effector
1252 contributes to quantitative resistance to the stem canker disease in *Brassica napus*. *New Phytol*
1253 231:1510–1524. <https://doi.org/10.1111/nph.17292>

1254 Johal GS, Briggs SP (1992) Reductase activity encoded by the HM1 disease resistance gene in maize.
1255 *Science* 258:985–987. <https://doi.org/10.1126/science.1359642>

1256 Jumper J, Evans R, Pritzel A, et al (2021) Highly accurate protein structure prediction with AlphaFold.
1257 *Nature* 596:583–589. <https://doi.org/10.1038/s41586-021-03819-2>

1258 Kathe E, Quezada-Martinez D, Kathe EI, et al (2019) Interspecific hybridization for *Brassica* crop
1259 improvement. *Crop Breeding, Genet Genomics* 1: e190007.

1260 <https://doi.org/10.20900/cbgg20190007>

1261 Katche EI, Schierholt A, Becker HC, et al (2023) Fertility, genome stability, and homozygosity in a diverse
1262 set of resynthesized rapeseed lines. *Crop J.* 11:468-477 <https://doi.org/10.1016/J.CJ.2022.07.022>

1263 Körber N, Wittkop B, Bus A, et al (2012) Seedling development in a *Brassica napus* diversity set and its
1264 relationship to agronomic performance. *Theor Appl Genet* 125:1275–1287.
1265 <https://doi.org/10.1007/s00122-012-1912-9>

1266 Kourelis J, Van Der Hoorn RAL (2018) Defended to the nines: 25 years of resistance gene cloning
1267 identifies nine mechanisms for R protein function. *Plant Cell* 30:285–299.
1268 <https://doi.org/10.1105/tpc.17.00579>

1269 Kruijt M, De Kock MJD, De Wit PJGM (2005) Receptor-like proteins involved in plant disease resistance.
1270 *Mol Plant Pathol* 6:85–97. <https://doi.org/10.1111/J.1364-3703.2004.00264.X>

1271 Lacoste L, Louvet J, Anselme C, et al (1969) Rôle de *Phoma lingam* (Tode) Desm. et de sa forme parfaite,
1272 *Leptosphaeria maculans* (Desm.) Ces. et de Not. dans les épidémies de nécrose du collet de colza
1273 (*Brassica napus* L. var. *oleifera* Metzger). *CR Acad Agric Fr* 55:981–987

1274 Larkan NJ, Lydiate DJ, Parkin IAP, et al (2013) The *Brassica napus* blackleg resistance gene *LepR3*
1275 encodes a receptor-like protein triggered by the *Leptosphaeria maculans* effector AVRML1. *New*
1276 *Phytol* 197:595–605. <https://doi.org/10.1111/nph.12043>

1277 Larkan NJ, Lydiate DJ, Yu F, et al (2014) Co-localisation of the blackleg resistance genes *Rlm2* and *LepR3*
1278 on *Brassica napus* chromosome A10. *BMC Plant Biol* 14:1–9. [https://doi.org/10.1186/s12870-](https://doi.org/10.1186/s12870-014-0387-z)
1279 [014-0387-z](https://doi.org/10.1186/s12870-014-0387-z)

1280 Larkan NJ, Ma L, Borhan MH (2015) The *Brassica napus* receptor-like protein RLM2 is encoded by a
1281 second allele of the *LepR3/Rlm2* blackleg resistance locus. *Plant Biotechnol J* 13:983–992.
1282 <https://doi.org/10.1111/pbi.12341>

1283 Larkan NJ, Ma L, Haddadi P, et al (2020) The *Brassica napus* wall-associated kinase-like (WAKL) gene
1284 *Rlm9* provides race-specific blackleg resistance. *Plant J* 104:892–900.
1285 <https://doi.org/10.1111/tpj.14966>

1286 Larkan NJ, Raman H, Lydiate DJ, et al (2016) Multi-environment QTL studies suggest a role for cysteine-
1287 rich protein kinase genes in quantitative resistance to blackleg disease in *Brassica napus*. *BMC*
1288 *Plant Biol* 16:1–16. <https://doi.org/10.1186/s12870-016-0877-2>

1289 Lazar N, Mesarich CH, Petit-Houdenot Y, et al (2022) A new family of structurally conserved fungal
1290 effectors displays epistatic interactions with plant resistance proteins. *PLoS Pathog* 18:1–27.
1291 <https://doi.org/10.1371/journal.ppat.1010664>

1292 Leflon M, Brun H, Eber F, et al (2007) Detection, introgression and localization of genes conferring
1293 specific resistance to *Leptosphaeria maculans* from *Brassica rapa* into *B. napus*. *Theor Appl Genet*
1294 115:897–906. <https://doi.org/10.1007/s00122-007-0616-z>

- 1295 Li C.-X, Cowling WA (2003) Identification of a single dominant allele for resistance to blackleg in
 1296 *Brassica napus* "Surpass 400." Plant Breed 122:485–488. <https://doi.org/10.1111/j.1439->
 1297 0523.2003.00863.x
- 1298 Li H, Kuo J, Barbetti MJ, Sivasithamparam K (2007) Differences in the responses of stem tissues of
 1299 spring-type *Brassica napus* cultivars with polygenic resistance and single dominant gene-based
 1300 resistance to inoculation with *Leptosphaeria maculans*. Can J Bot 85:191–203.
 1301 <https://doi.org/10.1139/B06-159>
- 1302 Li H, Sivasithamparam K, Barbetti MJ, Kuo J (2004) Germination and invasion by ascospores and
 1303 pycnidiospores of *Leptosphaeria maculans* on spring-type *Brassica napus* canola varieties with
 1304 varying susceptibility to blackleg. J Gen Plant Pathol 70:261–269.
 1305 <https://doi.org/10.1007/s10327-004-0125-8>
- 1306 Li Q, Wang B, Yu J, & Dou D (2021) Pathogen-informed breeding for crop disease resistance. J Integr
 1307 Plant Biol 63(2):305-311. <https://doi.org/10.1111/jipb.13029>
- 1308 Lo Presti L, Lanver D, Schweizer G, et al (2015) Fungal effectors and plant susceptibility. Annu Rev Plant
 1309 Biol 66:513–545. <https://doi.org/10.1146/annurev-arplant-043014-114623>
- 1310 Lowe RGT, Cassin A, Grandaubert J, et al (2014) Genomes and transcriptomes of partners in plant-
 1311 fungal- interactions between canola (*Brassica napus*) and two *Leptosphaeria* species. PLoS One
 1312 9:e103098. <https://doi.org/10.1371/journal.pone.0103098>
- 1313 Marcroft SJ, Elliott VL, Cozijnsen AJ, et al (2012) Identifying resistance genes to *Leptosphaeria maculans*
 1314 in Australian *Brassica napus* cultivars based on reactions to isolates with known avirulence
 1315 genotypes. Crop Pasture Sci 63:338. <https://doi.org/10.1071/cp11341>
- 1316 Mason AS, Huteau V, Eber F, et al (2010) Genome structure affects the rate of autosynthesis and
 1317 allosynthesis in AABC, BBAC and CCAB *Brassica* interspecific hybrids. Chromosom Res 18:655–666.
 1318 <https://doi.org/10.1007/s10577-010-9140-0>
- 1319 Mason AS, Snowdon RJ (2016) Oilseed rape: learning about ancient and recent polyploid evolution
 1320 from a recent crop species. Plant Biol. 18:883–892. <https://doi.org/10.1111/plb.12462>
- 1321 McCallum CM, Comai L, Greene EA, Henikoff S (2000) Targeted screening for induced mutations. Nat
 1322 Biotechnol 18:455–457. <https://doi.org/10.1038/74542>
- 1323 McDonald BA, Linde C (2002) Pathogen population genetics, evolutionary potential and durable
 1324 resistance. Annu Rev Phytopathol 40:349–379.
 1325 <https://doi.org/10.1146/annurev.phyto.40.120501.101443>
- 1326 McGee DC, Petrie GA (1978) Variability of *Leptosphaeria maculans* in relation to blackleg of oilseed
 1327 rape. Phytopathology 68:625–630. <https://doi.org/10.1094/Phyto-68-625>
- 1328 McNabb WM, Berg CGJ van den, Rimmer SR (1993) Comparison of inoculation methods for selection
 1329 of plants resistant to *Leptosphaeria maculans* in *Brassica napus*. Can J Plant Sci 73:1199–1207.

- 1330 <https://doi.org/10.4141/cjps93-159>
- 1331 Mendes-Pereira E, Balesdent M-H, Brun H, Rouxel T (2003) Molecular phylogeny of the *Leptosphaeria*
1332 *maculans*-*L. biglobosa* species complex. Mycol Res 107:1287–1304.
1333 <https://doi.org/10.1017/S0953756203008554>
- 1334 Mengistu A, Rimmer SR, Williams P. (1993) Protocols for in vitro sporulation, ascospore release, sexual
1335 mating, and fertility in crosses of *Leptosphaeria maculans*. Plant Dis 77:538–540.
1336 <https://doi.org/10.1094/PD-77-0538>
- 1337 Mengistu A, Rimmer SR, Williams PH (1991) Pathogenicity grouping of isolates of *Leptosphaeria*
1338 *maculans* on *Brassica napus* cultivars and their disease reaction profiles on rapid-cycling Brassicas.
1339 Plant Dis 1279–1282. <https://doi.org/10.1094/PD-75-1279>
- 1340 Mithen RF, Lewis BG, Heaney RK, Fenwick GR (1987) Resistance of leaves of *Brassica* species to
1341 *Leptosphaeria maculans*. Trans Br Mycol Soc 88:525–531. [https://doi.org/10.1016/s0007-](https://doi.org/10.1016/s0007-1536(87)80036-0)
1342 [1536\(87\)80036-0](https://doi.org/10.1016/s0007-1536(87)80036-0)
- 1343 Müller E, Tomasevic M (1957) Kulturversuche mit einigen Arten der Gattung *Leptosphaeria* Ces. et de
1344 Not. Phytopathol Z 29:287–294
- 1345 Navabi Z-K, Huebert T, Sharpe AG, et al (2013) Conserved microstructure of the *Brassica* B Genome of
1346 *Brassica nigra* in relation to homologous regions of *Arabidopsis thaliana*, *B. rapa* and *B. oleracea*.
1347 BMC Genomics 14:250. <https://doi.org/https://doi.org/10.1186/1471-2164-14-250>
- 1348 Navabi ZK, Stead KE, Pires JC, et al (2011) Analysis of B-genome chromosome introgression in
1349 interspecific hybrids of *Brassica napus* x *B. carinata*. Genetics 187:659–673.
1350 <https://doi.org/10.1534/genetics.110.124925>
- 1351 Neik TX, Ghanbarnia K, Ollivier B, et al (2022) Two independent approaches converge to the cloning of
1352 a new *Leptosphaeria maculans* avirulence effector gene, *AvrLmS-Lep2*. Mol Plant Pathol 23:733–
1353 748. <https://doi.org/10.1111/mpp.13194>
- 1354 Newman PL (1984) Differential host-parasite interactions between oilseed rape and *Leptosphaeria*
1355 *maculans*, the causal fungus of stem canker. Plant Pathol 33:205-210
- 1356 Newman PL, Bailey DJ (1987) Screening for resistance to canker (*Leptosphaeria maculans*) in winter
1357 oilseed rape (*Brassica napus* ssp. *oleifera*). Plant Pathol 36:346–354.
1358 <https://doi.org/10.1111/j.1365-3059.1987.tb02242.x>
- 1359 Niks RE, Qi X, Marcel TC (2015) Quantitative resistance to biotrophic filamentous plant pathogens:
1360 concepts, misconceptions, and mechanisms. Annu Rev Phytopathol 53: 445-470.
1361 <https://doi.org/10.1146/annurev-phyto-080614-115928>
- 1362 Noel K, Qi A, Gajula LH, et al (2022) Influence of elevated temperatures on resistance against phoma
1363 stem canker in oilseed rape. Front Plant Sci 13:6. <https://doi.org/10.3389/fpls.2022.785804>
- 1364 Outram MA, Figueroa M, Sperschneider J, et al (2022) Seeing is believing: Exploiting advances in

1365 structural biology to understand and engineer plant immunity. *Curr Opin Plant Biol* 67:102210
1366 This. <https://doi.org/10.1016/j.pbi.2022.102210>

1367 Parlange F, Daverdin G, Fudal I, et al (2009) *Leptosphaeria maculans* avirulence gene *AvrLm4-7* confers
1368 a dual recognition specificity by the *Rlm4* and *Rlm7* resistance genes of oilseed rape, and
1369 circumvents *Rlm4*-mediated recognition through a single amino acid change. *Mol Microbiol*
1370 71:851–863. <https://doi.org/10.1111/j.1365-2958.2008.06547.x>

1371 Peres A, Poisson B, Maisonneuve C (1996) Phoma du colza. Comment le champignon progresse dans
1372 la plante? *Oleoscope* 10–12

1373 Petit-Houdenot Y, Degrave A, Meyer M, et al (2019) A two genes – for – one gene interaction between
1374 *Leptosphaeria maculans* and *Brassica napus*. *New Phytol* 223:397–411.
1375 <https://doi.org/10.1111/nph.15762>

1376 Petrie G (1969) Variability in *Leptosphaeria maculans* (Desm.) Ces. et de Not., the cause of blackleg of
1377 rape. University of Saskatchewan, Canada

1378 Pilet-Nayel ML, Moury B, Caffier V, et al (2017) Quantitative resistance to plant pathogens in
1379 pyramiding strategies for durable crop protection. *Front Plant Sci* 8:1838.
1380 <https://doi.org/10.3389/fpls.2017.01838>

1381 Plissonneau C, Blaise F, Ollivier B, et al (2017) Unusual evolutionary mechanisms to escape effector-
1382 triggered immunity in the fungal phytopathogen *Leptosphaeria maculans*. *Mol Ecol* 26:2183–
1383 2198. <https://doi.org/10.1111/mec.14046>

1384 Plissonneau C, Daverdin G, Ollivier B, et al (2016) A game of hide and seek between avirulence genes
1385 *AvrLm4-7* and *AvrLm3* in *Leptosphaeria maculans*. *New Phytol* 209:1613–1624.
1386 <https://doi.org/10.1111/nph.13736>

1387 Plissonneau C, Rouxel T, Chèvre AM, et al (2018) One gene-one name: the *AvrLmJ1* avirulence gene of
1388 *Leptosphaeria maculans* is *AvrLm5*. *Mol Plant Pathol* 19:1012–1016.
1389 <https://doi.org/10.1111/mpp.12574>

1390 Quezada-Martinez D, Addo Nyarko CP, Schiessl S V., Mason AS (2021) Using wild relatives and related
1391 species to build climate resilience in *Brassica* crops. *Theor Appl Genet* 134:1711–1728.
1392 <https://doi.org/10.1007/s00122-021-03793-3>

1393 Rahman MH, Hawkins G, Avery M, et al (2007) Introgression of blackleg (*Leptosphaeria maculans*)
1394 resistance into *Brassica napus* from *B. carinata* and identification of microsatellite (SSR) markers.
1395 In: Proceedings of 12th International Rapeseed Congress. Wuhan, China, pp 47–50

1396 Raman H, Raman R, Coombes N, et al (2016) Genome-wide Association Study identifies new loci for
1397 resistance to *Leptosphaeria maculans* in canola. *Front Plant Sci* 7:1513.
1398 <https://doi.org/10.3389/fpls.2016.01513>

1399 Raman H, Raman R, Qiu Y, et al (2021) The *Rlm13* gene, a new player of *Brassica napus*–*Leptosphaeria*

1400 *maculans* interaction maps on chromosome C03 in canola. *Front Plant Sci* 12:654604–654604.
1401 <https://doi.org/10.3389/fpls.2021.654604>

1402 Rimmer SR (2006) Resistance genes to *Leptosphaeria maculans* in *Brassica napus*. *Can J Plant Pathol*
1403 28:S288–S297. <https://doi.org/10.1080/07060660609507386>

1404 Rimmer SR, Van Den Berg CGJ (1992) Resistance of oilseed *Brassica* spp. to blackleg caused by
1405 *Leptosphaeria maculans*. *Can J Plant Pathol* 14:56–66.
1406 <https://doi.org/10.1080/07060669209500906>

1407 Robin AHK, Larkan NJ, Laila R, et al (2017) Korean *Brassica oleracea* germplasm offers a novel source
1408 of qualitative resistance to blackleg disease. *Eur J Plant Pathol* 149:611–623.
1409 <https://doi.org/10.1007/s10658-017-1210-0>

1410 Röhrig L, Dussart F (2022) Does abiotic host stress favour Dothideomycete-induced disease
1411 development? *Plants* 11:1615. <https://doi.org/10.3390/plants11121615>

1412 Rouxel T, Balesdent M-H (2005) The stem canker (blackleg) fungus, *Leptosphaeria maculans*, enters
1413 the genomic era. *Mol Plant Pathol* 6:225–241. <https://doi.org/10.1111/j.1364-3703.2005.00282.x>

1415 Rouxel T, Balesdent M-H (2017) Life, death and rebirth of avirulence effectors in a fungal pathogen of
1416 *Brassica* crops, *Leptosphaeria maculans*. *New Phytol* 214:526–532.
1417 <https://doi.org/10.1111/nph.14411>

1418 Rouxel T, Balesdent M-H (2013) From model to crop plant-pathogen interactions: Cloning of the first
1419 resistance gene to *Leptosphaeria maculans* in *Brassica napus*. *New Phytol* 197:356–358.
1420 <https://doi.org/10.1111/nph.12099>

1421 Rouxel T, Grandaubert J, Hane JK, et al (2011) Effector diversification within compartments of the
1422 *Leptosphaeria maculans* genome affected by repeat-induced point mutations. *Nat Commun* 2:1–
1423 10. <https://doi.org/10.1038/ncomms1189>

1424 Rouxel T, Penaud A, Pinochet X, et al (2003a) A 10-year survey of populations of *Leptosphaeria*
1425 *maculans* in France indicates a rapid adaptation towards the *Rlm1* resistance gene of oilseed rape.
1426 *Eur J Plant Pathol* 109:871–881. <https://doi.org/10.1023/A:1026189225466>

1427 Rouxel T, Willner E, Coudard L, Balesdent M-H (2003b) Screening and identification of resistance to
1428 *Leptosphaeria maculans* (stem canker) in *Brassica napus* accessions. *Euphytica* 133:219–231.
1429 <https://doi.org/10.1023/A:1025597622490>

1430 Roy NN, Reeves J (1975) Breeding better rape and linseed for Western Australia. *J Dep Agric West Aust*
1431 Ser 4 16:93–97

1432 Saintenac C, Lee WS, Cambon F, et al (2018) Wheat receptor-kinase-like protein Stb6 controls gene-
1433 for-gene resistance to fungal pathogen *Zymoseptoria tritici*. *Nat Genet* 50:368–374.
1434 <https://doi.org/10.1038/s41588-018-0051-x>

1435 Sánchez-Vallet A, Fouché S, Fudal I, et al (2018) The genome biology of effector gene evolution in
1436 filamentous plant pathogens. *Annu Rev Phytopathol* 56:21–40.
1437 <https://doi.org/10.1146/annurev-phyto-080516-035303>

1438 Seong K, Krasileva K V (2023) Prediction of effector protein structures from fungal phytopathogens
1439 enables evolutionary analyses. *Nat Microbiol* 8:174–187. [https://doi.org/10.1038/s41564-022-](https://doi.org/10.1038/s41564-022-01287-6)
1440 [01287-6](https://doi.org/10.1038/s41564-022-01287-6)

1441 Seyis F, Snowdon RJ, Lühs W, Friedt W (2003) Molecular characterization of novel resynthesized
1442 rapeseed (*Brassica napus*) lines and analysis of their genetic diversity in comparison with spring
1443 rapeseed cultivars. *Plant Breed* 122:473–478. [https://doi.org/10.1111/j.1439-](https://doi.org/10.1111/j.1439-0523.2003.00859.x)
1444 [0523.2003.00859.x](https://doi.org/10.1111/j.1439-0523.2003.00859.x)

1445 Shoemaker RA, Brun H (2001) The teleomorph of the weakly aggressive segregate of *Leptosphaeria*
1446 *maculans*. *Can J Bot* 79:412–419. <https://doi.org/10.1139/cjb-79-4-412>

1447 Sivasithamparam K, Barbetti MJ, Li H (2005) Recurring challenges from a necrotrophic fungal plant
1448 pathogen: A case study with *Leptosphaeria maculans* (causal agent of blackleg disease in
1449 Brassicas) in Western Australia. *Ann Bot* 96:363–377. <https://doi.org/10.1093/aob/mci194>

1450 Smith HC (1956) *Leptosphaeria napi*, the perithecial form of *Phoma lingam* causing dry-rot disease of
1451 Brassicas. *New Zeal Sci Rev* 14:116–117

1452 Smith HC, Sutton BC (1964) *Leptosphaeria maculans* the ascogenous state of *Phoma lingam*. *Trans Br*
1453 *Mycol Soc* 47:159-IN1. [https://doi.org/10.1016/s0007-1536\(64\)80049-8](https://doi.org/10.1016/s0007-1536(64)80049-8)

1454 Song JM, Guan Z, Hu J, et al (2020) Eight high-quality genomes reveal pan-genome architecture and
1455 ecotype differentiation of *Brassica napus*. *Nat Plants* 6:34–45. [https://doi.org/10.1038/s41477-](https://doi.org/10.1038/s41477-019-0577-7)
1456 [019-0577-7](https://doi.org/10.1038/s41477-019-0577-7)

1457 Song JM, Liu DX, Xie WZ, et al (2021) BnPIR: *Brassica napus* pan-genome information resource for 1689
1458 accessions. *Plant Biotechnol J* 19:412–414. <https://doi.org/10.1111/pbi.13491>

1459 Sprague SJ, Marcroft SJ, Hayden HL, Howlett BJ (2006) Major gene resistance to blackleg in *Brassica*
1460 *napus* overcome within three years of commercial production in Southeastern Australia. *Plant*
1461 *Dis* 90:190–198. <https://doi.org/10.1094/PD-90-0190>

1462 Stachowiak A, Olechnowicz J, Jedryczka M, et al (2006) Frequency of avirulence alleles in field
1463 populations of *Leptosphaeria maculans* in Europe. *Eur J Plant Pathol* 114:67–75.
1464 <https://doi.org/10.1007/s10658-005-2931-z>

1465 Stam R, McDonald BA (2018) When resistance gene pyramids are not durable—the role of pathogen
1466 diversity. *Mol Plant Pathol* 19:521–524. <https://doi.org/10.1111/mpp.12636>

1467 Struss D, Bellin U, Röbbelen G (1991) Development of B-Genome chromosome addition lines of *B.*
1468 *napus* using different interspecific *Brassica* hybrids. *Plant Breed* 106:209–214.
1469 <https://doi.org/10.1111/j.1439-0523.1991.tb00503.x>

- 1470 Struss D, Quiros CF, Plieske J, Röbbelen G (1996) Construction of *Brassica* B genome syntenic groups
1471 based on chromosomes extracted from three different sources by phenotypic, isozyme and
1472 molecular markers. *Theor Appl Genet* 93:1026–1032. <https://doi.org/10.1007/BF00230120>
- 1473 Talbi N, Fokkens L, Audran C, Petit-Houdenot Y, Blaise F, Gay EJ, Gautier A, Rouxel T, Balesdent M-H,
1474 Rep M, Fudal I (2023) The neighboring genes *AvrLm10A* and *AvrLm10B* are part of a large
1475 multigene family of cooperating effector genes conserved in Dothideomycetes and
1476 Sordariomycetes. *Mol Plant Pathol*. 24:914-931. <https://doi.org/10.1111/mpp.13338>
- 1477 Tang D, Wang G, Zhou JM. Receptor Kinases in Plant-Pathogen Interactions: More Than Pattern
1478 Recognition. *Plant Cell*. 2017 29(4):618-637. doi: 10.1105/tpc.16.00891.
- 1479 Thurling N, Venn LA (1977) Variation in the responses of rapeseed (*Brassica napus* and *B. campestris*)
1480 cultivars to blackleg (*Leptosphaeria maculans*) infection. *Aust J Exp Agric* 17:445–451.
1481 <https://doi.org/10.1071/EA9770445>
- 1482 Tirnaz S, Bayer PE, Inturrisi F, et al (2020) Resistance gene analogs in the Brassicaceae: identification,
1483 characterization, distribution, and evolution. *Plant Physiol* 184:909–922.
1484 <https://doi.org/10.1104/PP.20.00835>
- 1485 Toscano-Underwood C, Huang YJ, Fitt BDL, Hall AM (2003) Effects of temperature on maturation of
1486 pseudothecia of *Leptosphaeria maculans* and *L. biglobosa* on oilseed rape stem debris. *Plant*
1487 *Pathol* 52:726–736. <https://doi.org/10.1111/j.1365-3059.2003.00930.x>
- 1488 Tulasne LR, Tulasne C (1863) *Selecta fungorum carpologia*. Vol 2, La Presse Impériale, Paris, 300 pp
- 1489 U N (1935) Genome analysis in *Brassica* with special reference to the experimental formation of *B.*
1490 *napus* and peculiar mode of fertilization. *Jpn J Bot* 7:389–452
- 1491 Van de Wouw AP, Howlett BJ (2020) Advances in understanding the *Leptosphaeria maculans* - *Brassica*
1492 pathosystem and their impact on disease management. *Can J Plant Pathol* 42:149–163.
1493 <https://doi.org/10.1080/07060661.2019.1643788>
- 1494 Van de Wouw AP, Lowe RGT, Elliott CE, et al (2014) An avirulence gene, *AvrLmJ1*, from the blackleg
1495 fungus, *Leptosphaeria maculans*, confers avirulence to *Brassica juncea* cultivars. *Mol Plant Pathol*
1496 15:523–530. <https://doi.org/10.1111/mpp.12105>
- 1497 Van De Wouw AP, Marcroft SJ, Barbetti MJ, et al (2009) Dual control of avirulence in *Leptosphaeria*
1498 *maculans* towards a *Brassica napus* cultivar with “sylvestris-derived” resistance suggests
1499 involvement of two resistance genes. *Plant Pathol* 58:305–313. <https://doi.org/10.1111/j.1365-3059.2008.01982.x>
- 1500
- 1501 Van de Wouw AP, Marcroft SJ, Sprague SJ, et al (2021) Epidemiology and management of blackleg of
1502 canola in response to changing farming practices in Australia. *Australas Plant Pathol* 50:137–149.
1503 <https://doi.org/10.1007/s13313-020-00767-9>
- 1504 Van de Wouw AP, Sheedy EM, Ware AH, et al (2022) Independent breakdown events of the *Brassica*

1505 *napus Rlm7* resistance gene including via the off-target impact of a dual-specificity avirulence
1506 interaction. *Mol Plant Pathol* 23:997–1010. <https://doi.org/10.1111/mpp.13204>

1507 Vincenot L, Balesdent M-H, Li H, et al (2008) Occurrence of a new subclade of *Leptosphaeria biglobosa*
1508 in Western Australia. *Phytopathology* 98:321–329. <https://doi.org/10.1094/PHYTO-98-3-0321>

1509 West JS, Biddulph JE, Fitt BDL, Gladders P (1999) Epidemiology of *Leptosphaeria maculans* in relation
1510 to forecasting stem canker severity on winter oilseed rape in the UK. *Ann Appl Biol* 135:535–546.
1511 <https://doi.org/10.1111/j.1744-7348.1999.tb00885.x>

1512 West JS, Kharbanda PD, Barbetti MJ, Fitt BDL (2001) Epidemiology and management of *Leptosphaeria*
1513 *maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe. *Plant Pathol*
1514 50:10–27. <https://doi.org/10.1046/j.1365-3059.2001.00546.x>

1515 Yang C, Zou Z, Fernando WGD (2021) The effect of temperature on the hypersensitive response (Hr) in
1516 the *Brassica napus–Leptosphaeria maculans* pathosystem. *Plants* 10:843.
1517 <https://doi.org/10.3390/plants10050843>

1518 Yang Z, Wang S, Wei L, et al (2023) BnLR: A multi-omics database with various tools for *Brassica napus*
1519 research and breeding. *Mol Plant*. <https://doi.org/10.1016/J.MOLP.2023.03.007>

1520 Yu F, Gugel RK, Kutcher HR, et al (2013) Identification and mapping of a novel blackleg resistance locus
1521 *LepR4* in the progenies from *Brassica napus* × *B. rapa* subsp. *sylvestris*. *Theor Appl Genet*
1522 126:307–315. <https://doi.org/10.1007/s00122-012-1919-2>

1523 Yu F, Lydiate DJ, Rimmer SR (2005) Identification of two novel genes for blackleg resistance in *Brassica*
1524 *napus*. *Theor Appl Genet* 110:969–979. <https://doi.org/10.1007/s00122-004-1919-y>

1525 Yu F, Lydiate DJ, Rimmer SR (2008) Identification and mapping of a third blackleg resistance locus in
1526 *Brassica napus* derived from *B. rapa* subsp. *sylvestris*. *Genome* 51:64–72.
1527 <https://doi.org/10.1139/G07-103>

1528 Zhang X, Fernando WGD (2018) Insights into fighting against blackleg disease of *Brassica napus* in
1529 Canada. *Crop Pasture Sci* 69:40–47. <https://doi.org/10.1071/CP16401>

1530 Zhang X, Peng G, Kutcher HR, et al (2016) Breakdown of *Rlm3* resistance in the *Brassica napus–*
1531 *Leptosphaeria maculans* pathosystem in western Canada. *Eur J Plant Pathol* 145:659–674.
1532 <https://doi.org/10.1007/s10658-015-0819-0>

1533 Zheng X, Koopmann B, Ulber B, von Tiedemann A (2020) A global survey on diseases and pests in
1534 oilseed rape—current challenges and innovative strategies of control. *Front Agron* 2:590908.
1535 <https://doi.org/10.3389/fagro.2020.590908>

1536 **Statements & Declarations**

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1543 *Competing interest*

1544 The authors declare no conflict of interest.

1545 Author contributions

1546 ALM had the idea for the review, all authors performed the literature search, drafted and revised the
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