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REVIEW ARTICLE

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Current progress in studying blackleg disease (*Leptosphaeria maculans* and *L. biglobosa*) of canola in Iran: Where do we stand now?

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Abstract

Blackleg, caused by a complex of *Leptosphaeria* species (*L. maculans* and *L. biglobosa*), is a fungal disease on *Brassica* species, especially important in canola (*Brassica napus*). Since the first report of *L. biglobosa* in Iran in 2007 and *L. maculans* in 2008, both species are now of major importance in Iran affecting 10 provinces and 30 regions, with a higher prevalence in the northern provinces of Mazandaran and Golestan. Despite the rapid progression of the disease and the emergence of new *Leptosphaeria* races in Iran, the research into this pathogen has not progressed at the same rate and is limited to phenotypic characterization studies, pathogenicity research, and to a lesser extent, disease management research. Given the rapid increase in canola cultivation in Iran and changes in the genetic diversity of the pathogen populations, it is likely that blackleg disease will increasingly become a severe threat to Iran's canola production. Therefore, systematic and prospective studies, along with fundamental research on the pathogen's biology, epidemiology, and genetic diversity, would provide critical information for the development of disease management strategies. Here, we review the research that has been carried out to date on blackleg disease in Iran and describe the extent of progress towards disease control, especially in disease-prone regions.

KEYWORDS
blackleg, canola, disease management, *Leptosphaeria maculans*

1 | BACKGROUND

Oilseed rape (*Brassica napus*) is an allotetraploid species formed as a result of interspecific hybridization between the two diploid species *B. oleracea* and *B. rapa* (Morinaga, 1934). In 1961, Dr Baldur R. Stefansson from the Department of Plant Science, University of Manitoba, modified the taste, colour, and high concentration of erucic acid in *B. napus* and *B. rapa* cultivars, used primarily for industrial applications, making them suitable for human consumption (Raymer, 2002). In 1967, seeds from the Polish cultivar Bronowski were found to have a low concentration of glucosinolates and this genetic resource was used to develop cultivars with low erucic acid and low glucosinolates. In 1974 Dr Stefansson, with Dr Keith Downey from Agriculture and Agri-Food Canada, registered the first canola ("CAN" for Canada and "OLA" for Oil, Low Acid) variety of its kind, Tower.

2 | CANOLA CULTIVATION IN IRAN

Canola plays an important role in providing edible oil for human consumption and is Iran's most cultivated oilseed crop. It has been grown for two decades in Iran, since it was imported from Australia. Canola is a temperate crop and depending on the region's climatic

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conditions, winter and spring canola types can be sown during autumn. In Iran's north and north-west regions, canola is grown as a rainfall crop, while in the western, central, and south-western regions, it is an irrigated crop (Figure 1). More than 40% of the canola-growing area in Iran is concentrated in the provinces of Golestan (93,384 ha) and Mazandaran (24,604 ha), which are situated in the north of the country (Figure 2; Ahmadi et al., 2019), where the minimum precipitation is slightly more than 400 mm/year. Khuzestan and Ilam, located in the south-west and west of Iran, are the other major canola producers, with cultivation areas of 32,000 and 10,515 ha respectively (Figure 2). Generally, canola yield varies from 2 t/ha for spring-type canola in northern regions of Iran, to >3 t/ha for winter-type canola in the country's western parts. The annual canola production in Iran was estimated to be about 420,000 t in 2017–2018, and an additional 90,000 t was imported (Zamanmirabadi, 2020).

Although only a narrow range of canola cultivars was initially cultivated in Iran, experiments were undertaken to develop and introduce new cultivars into practice. In recent years, the Ministry of Agriculture of Iran has increased efforts to import new hybrids from Germany, Canada, France, and Australia, as well as introduce

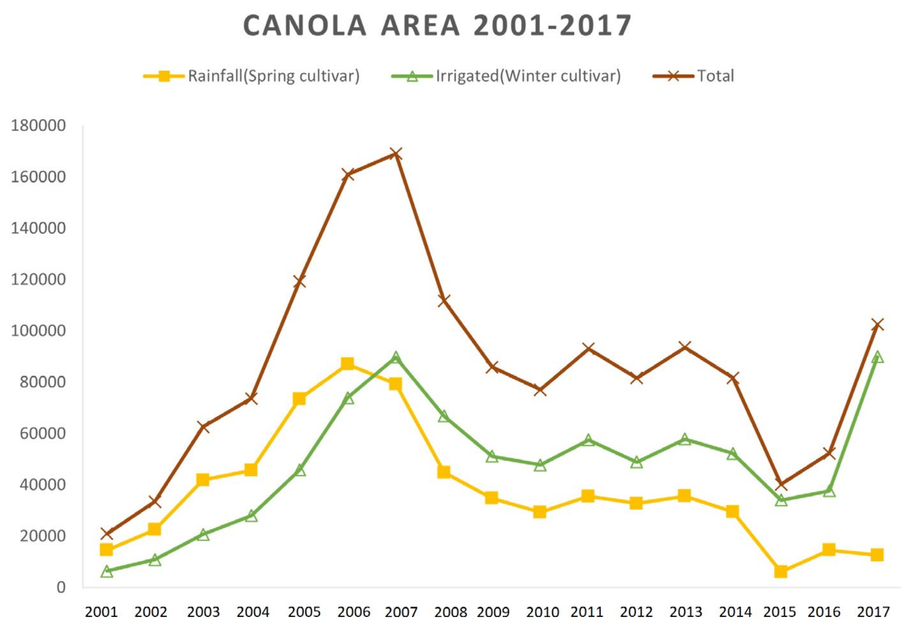

FIGURE 1 Canola area (ha) in Iran under rainfall and irrigated conditions during 2001–2017 [Colour figure can be viewed at wileyonlinelibrary.com]

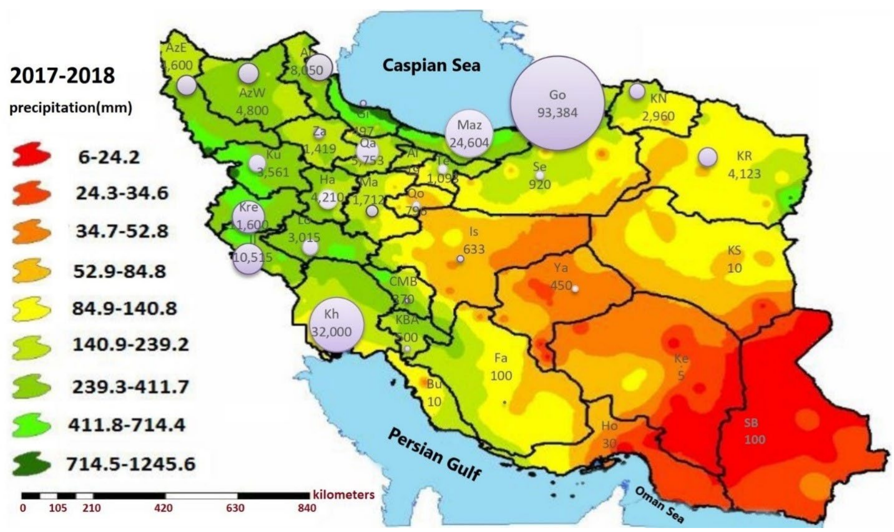

FIGURE 2 Canola planting areas and precipitation rate (mm) in different regions of Iran in 2017–2018 (Iran Meteorological Organization). The size of the grey circle represents the canola cultivation area (ha) in the different provinces: Alborz (Al), Ardabil (Ar), Azerbaijan East (AzE), Azerbaijan West (AzW), Bushehr (Bu), Chahar Mahaal and Bakhtiari (CMB), Fars (Fa), Gilan (Gi), Golestan (Go), Hamadan (Ha), Hormozgān (Ho), Ilam (Il), Isfahan (Is), Kerman (Ke), Kermanshah (Kre), Khorasan North (KN), Khorasan Razavi (KR), Khorasan South (KS), Khuzestan (Kh), Kohgiluyeh and Boyer-Ahmad (KBA), Kurdistan (Ku), Lorestan (Lo), Markazi (Ma), Mazandaran (Maz), Qazvin (Qa), Qom (Qo), Semnan (Se), Sistan and Baluchestan (SB), Tehran (Te), Yazd (Ya), Zanjan (Za) [Colour figure can be viewed at wileyonlinelibrary.com]

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TABLE 1 The most cultivated canola cultivars in Iran

Cultivar	Country of origin	Type	Registration date in Iran	Yield (t/ha)	Blackleg rating	References
Hyola 50	Australia	Spring	2009	2.0–2.5	MR	Abbaspour (2020)
Hyola 420	Australia	Spring	2001	2.3–2.8	ND	
Hyola 401	Australia	Spring	2000	2.2–2.7	S	Zamanmirabadi, Rahnama, et al. (2010)
Hyola 4815	Australia	Spring	2009	2.2–2.7	MR	Abbaspour (2020)
Okapi	France	Winter	2001	3.5–4.5	S	Zamanmirabadi, Rahnama, et al. (2010)
Agamax	Germany	Winter	2019	3.5–4.0	ND	
Trapper	Germany	Winter	2019	3.5–4.0	ND	
RGS003	Germany	Spring	2004	2.1–2.6	S	Keypoor et al. (2015)
Nima	Iran	Winter	2016	3.5–4.0	ND	
Ahmadi	Iran	Winter	2014	3.5–4.0	MS	Rahmanpour and Alizadeh (2015)
Opera	Iran	Winter	2003	3.4–3.9	ND	
Zafar	Iran	Spring	2012	2.5–3.1	MS	Zamanmirabadi, Rahnama, et al. (2010)
Zaman	Iran	Spring	2016	2.2–2.7	MS	Abbaspour (2020)
Mahtab	Iran	Spring	2016	2.2–2.7	MS	Abbaspour (2020)
Delgan	Iran	Spring	2014	2.2–2.7	ND	

Abbreviations: MR, moderately resistant; MS, moderately susceptible; ND, not determined; S, susceptible.

new Iranian cultivars. Since 2000, a broad range of canola cultivars has been cultivated in Iran (Table 1); during 2000–2017, the most cultivated cultivar was the hybrid Hyola 401 (Oilseeds Research & Development Company, 2018).

3 | CANOLA PRODUCTION LIMITING FACTORS

Canola production in Iran is affected by several biotic and abiotic stress factors. Among abiotic stresses, drought and salinity are the two major ones that decrease the yield and quality of canola seed and oil in Iran (Majidi et al., 2015; Moradbeigi et al., 2019; Shamsodin, 2006; Zamani et al., 2010). In Iran's north-west regions, cold spring damage is a further barrier affecting canola development (Aghel & Zoghi, 2009). Biotic factors including pests, diseases, and weeds influence canola growth, development, and yield worldwide (Chattopadhyay et al., 2015). In Iran, 26 insects belonging to six orders and 13 families have been shown to damage canola plants at different phenological stages. The major insect pests include flea beetles (*Phyllotreta corrugata*, *Psylliodespersicus*), aphids (*Brevicorynebrassicae*, *Lipaphis erysimi*, *Myzus persicae*), pollen beetles (*Meligethes aeneus*), and weevils (*Ceutorhynchus* spp.) (Keyhanian et al., 2005; Zamanmirabadi et al., 2011a). However, almost all these insects can be controlled by chemical and/or agronomic methods (Afshari-Azad, Dalili, et al., 2016; Barari, 2015; Zamanmirabadi, 2018). Wild mustard (*Sinapis arvensis*) and turnip weed (*Rapistrum rugosum*) are considered the most common and damaging weeds in Iran (Shimi et al., 2006; Zamanmirabadi et al., 2012).

Fungal diseases such as Alternaria leaf and pod spot (*Alternaria* spp.), downy mildew (*Hyaloperonospora parasitica*), blackleg (*Leptosphaeria maculans*), and white stem rot (*Sclerotinia sclerotiorum*) (Mehdi Alamdarlou et al., 2009; Zamanmirabadi et al., 2011b) are the major diseases on canola worldwide, including in Iran (Czeslaw et al., 2002). Among these fungal diseases, white stem rot and blackleg are the most severe threats to Iran's canola production (Zamanmirabadi et al., 2018a).

Blackleg is a disease caused by a complex of two *Leptosphaeria* species: *L. maculans* and *L. biglobosa*. Blackleg symptoms can be observed at all growth stages and in all plant parts, including cotyledons, leaves, stems, and siliques (Vakili-Zarj et al., 2017c; Zamanmirabadi, Rahnama, et al., 2010). The first symptoms of the disease appear on the leaves as grey-green spots. After leaf infection, fungal mycelia can extend from the leaves and petioles into the upper and basal stems. The fungus then breaks down the cortex cells to form stem cankers (Howlett et al., 2001), which are the main cause of yield loss. In Iran, losses from blackleg vary between different regions and years, although to date there has been no accurate estimation of the damage.

4 | FIELD AND PHENOTYPIC STUDIES

Canola has become increasingly popular in Iran due to its high yield, ability to perform efficiently under the climatic conditions and simplicity of its planting. However, blackleg is affecting the profitability. Favourable conditions for disease development and damage in the northern regions of Iran, along with the increase in planting area, have led to more studies on this fungus over the past 10 years, especially in the Mazandaran and Golestan provinces (Afshari-Azad et al., 2008; Afshari-Azad, Keihanian, et al., 2016; Ghasemi Alitapeh et al., 2018; Momeni & Afshari-Azad, 2010; Vakili-Zarj et al., 2017a, 2017b, 2017c; Zamanmirabadi,

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Mehdi-Alamdarlou, Esmaeilifar, Fathi, 2010; Zamanmirabadi, Rahnama, et al., 2010, 2016).

In 2006, a number of blackleg isolates were collected from canola stems grown in Golestan province and sent to the Blackleg Research Laboratory at the Department of Plant Science, University of Manitoba. Morphological and pathogenicity tests showed that the isolates were from the nonaggressive group, *L. biglobosa* (Fernando et al., 2007). Unofficially, the presence of *Leptosphaeria*, without any description of its characteristics, had been recorded in canola fields in the north of Iran (A. Zamanmirabadi, unpublished observations in 2005; Mehdi Alamdarlou, Zamanmirabadi, Esmaeilifar, 2007). However, due to difficulties in distinguishing similar species in their asexual form, the lack of availability of molecular assays for separating *Leptosphaeria* species, and the lack of considerable damage by this disease in farmers' fields, the presence of this fungus was not officially reported until the laboratory test in 2007 (Fernando et al., 2007). The sexual form of blackleg was subsequently observed on the cultivars Hyola 401 and Zarfam in the north of Iran (Zamanmirabadi et al., 2008). In another study in Fars province (Marvdasht region), undertaken to identify the fungal agents involved in canola crown and root rots, two isolates of *Phoma lingam* (anamorph of *L. maculans*) were reported (Heydari et al., 2010). Furthermore, in the south of Iran (Khuzestan province), a total of 38 *P. lingam* isolates were detected (Laraki & Farokhinejad, 2013).

4.1 | Distribution of *L. maculans*

Due to favourable environmental conditions for the blackleg pathogen in the north of Iran (Mazandaran and Golestan provinces), most research on *Leptosphaeria* spp. has focused on these areas. Initial identification of *L. maculans* and *L. biglobosa* was performed in 14 regions located in Mazandaran and Golestan provinces (Zamanmirabadi, Rahnama, et al., 2010), with an additional 11 provinces subsequently explored for the presence of the pathogen. The pathogen was detected in 16 regions in eight provinces: Qazvin, Lorestan, Hamadan, Fars, Kermanshah, Khoramabad, Ardebil, and Khuzestan (Afshari-Azad et al., 2008; Vakili-Zarj et al., 2017c; Zamanmirabadi, Mehdi-Alamdarlou, Esmaeilifar, Fathi, 2010). Currently, blackleg is present in 30 regions and 10 provinces in Iran. However, the sexual form of *Leptosphaeria* spp. has only been observed in three locations (Mazandaran, Golestan, and Lorestan provinces) (Zamanmirabadi, Mehdi-Alamdarlou, Esmaeilifar, Fathi, 2010). Whilst a considerable portion of winter-type canola and a small portion of spring-type canola are cultivated in Iran's western and southern regions, the lack of high humidity in cold regions and high temperatures in southern regions means that blackleg disease has not yet spread in these regions.

4.2 | The sexual form of *Leptosphaeria*

Although pycnidiospores can be considered a primary (Ghanbarnia et al., 2011) or secondary inoculum (Li et al., 2007), there is no information about the role of pycnidiospores in spreading the disease in Iran. Ascospores are the initial source of blackleg disease and play an essential role in spreading the disease (West et al., 2001; Zamanmirabadi et al., 2016). Ascospores of *Leptosphaeria* species form in pseudothecia, fruiting bodies of the sexual stage, generally on stubble from the previous growing season. Sexual reproduction is essential to maintaining genetic diversity (Barrins et al., 2002, 2004; Mahuku et al., 1997). Thus, investigation on the sexual reproduction of *L. maculans* would help in understanding the genetic structure of the pathogen (Brazauskiene et al., 2011) and provide information that could be used in epidemiological studies (Kruse & Verreet, 2005) and agronomic disease management approaches (Gladders et al., 2006).

In Iran, the sexual form of *P. lingam* was first reported from a mountainous region located in Kiasar village, south of Mazandaran province (Zamanmirabadi et al., 2008). In the north of Iran, spring-type canola is commonly sown from October to November and harvested in May. Farmers typically cultivate various crops, including rice, soybean, maize, and various vegetables, in most spring and summer seasons. Thus, given this crop rotation cycle and tillage implementation, few mature ascocarps (pseudothecia) are formed, impeding surveying the sexual form of *L. maculans* or *L. biglobosa* on stem residue-infected canola. Winter-type canola is planted in mountainous regions with cold weather (south of Mazandaran province, such as Kiasar and Hezargrib regions, and eastern Golestan province, such as Golidagh village), and is harvested from June to July. If there is no summer precipitation after harvesting, there will be no further planting of rotation crops. This planting strategy provides suitable conditions for maturing sexual forms of *Leptosphaeria* spp. on crops residues; therefore, more genetic diversity will be expected in these areas.

As has previously been demonstrated by Huang et al. (2001), using a combination of pathogenicity (Zamanmirabadi, Rahnama, & Esmaeilifar, 2009; Zamanmirabadi, Rahnama, et al., 2010) and morphological analyses (Zamanmirabadi et al., 2016), it has been shown that it is possible to distinguish Iranian populations of *L. maculans* and *L. biglobosa* using morphological features of germinated ascospores including number, size, shape, and origin of germ tubes. Through studying the dimensions of ascospores of different populations of *L. maculans* and *L. biglobosa* in the north of Iran, it has been shown that these ascospores were bigger than ascospores reported in other areas (Zamanmirabadi et al., 2016). The largest length and width of sexual spores were 85 and 12.5 μm, respectively, with variations in the range of length and width of 46.25 and 7.5 μm, respectively. Further results (Huang et al., 2001; Zamanmirabadi et al., 2016) also confirmed that *L. biglobosa* ascospore germ tubes were longer than those in *L. maculans*, and the average number of germ tubes produced by *L. maculans* ascospores was greater than that in *L. biglobosa*.

For a more detailed study of ascospore diversity, it would be most informative if a combination of morphological and molecular tests were undertaken to differentiate the characteristics of the ascospores based on the species and regions.

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4.3 | Host range

In Iran, the sexual form of *L. maculans* was first reported in fields where there was no recorded history of canola cultivation. Nevertheless, there was a noticeable presence of the fungus in the first recorded year of canola cultivation, with a frequency of 60% stem canker symptoms and <5% of plant lodging (Zamanmirabadi et al., 2008). Therefore, *Leptosphaeria* spp. may have one or more alternate hosts in the region. To date, wild mustard (*S. arvensis*) and turnip weed (*R. rugosum*) have been identified as alternative hosts for *Leptosphaeria* spp. in Iran (Zamanmirabadi, Rahnama, et al., 2010). However, other *Brassica* species could also host the fungus. Thus, comprehensive research should be undertaken to investigate this issue further. A broad range of hosts for the pathogen has previously been reported (Barbetti, 1978; Johnson & Lewis, 1994; Petrie, 1969). For example, pathogenicity groups 1 (PG1) and *L. biglobosa* isolates (nonaggressive) have been obtained from different mustard species in the north of Iran (Zamanmirabadi, Rahnama, et al., 2010). However, more field research is still required to find the most aggressive mustard isolates.

4.4 | Seed contamination

L. maculans can transmit physically through the seeds, and thus, infected seeds could be responsible for introducing the pathogen to new fields and lead to subsequent spread of the disease (Allen & Smith, 1961; Zhang et al., 2014). Although the volume of commercial imported canola seeds to Iran is not considerable, and *L. maculans* is a quarantined pathogen in imported seeds for Iran, there are no strict regulations for preventing or minimizing the introduction of the pathogen into the country. Out of 1769 fungal isolates collected from 160 seed samples from seed production fields in Khuzestan, Ardabil, Tehran, Hamadan and Khorasan, 16 isolates were detected as *P. lingam* (Zendehdel et al., 2008). In a further study to determine the level of seedborne fungal infections of canola seeds, 56 canola seed samples were collected from different canola-growing regions in Kermanshah province, where no samples were infected with *Leptosphaeria* spp. (Younesi et al., 2012). In 2013, a study was conducted to investigate the possibility of detecting canola seed infected with *Leptosphaeria* spp. (Zad et al., 2014). For this purpose, 26 canola fields with no-fungicide application in Golestan provinces (Kalaleh, Kordkuy, Aliabad-e-Katul) were studied. Before harvesting, diseased and healthy plants (a total of 500 seeds for each category) were selected for determining the incidence of pathogen infection based on International Seed Testing Association protocols (Anonymous, 1966). The results demonstrated that although *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp. and *Sclerotinia sclerotiorum* were visible, both healthy and diseased seeds lacked *P. lingam* contamination (Zad et al., 2014). However, in another study during 2014–2015 in Golestan province, canola seed contamination with *L. maculans* was confirmed using molecular and morphological methods (Vakili-Zarj et al., 2017c). Therefore, it is essential to use molecular markers to monitor imported seeds to ensure they are free of *L. maculans* or *L. biglobosa* and determine genetic similarity between domestic and imported populations.

4.5 | Pathogenicity research

Canola has a gene-for-gene interaction with *L. maculans*, where the product of a resistance gene (*R*) in the plant interacts with a corresponding avirulence gene (*Avr*) product in the pathogen (Ansan-Melayah et al., 1998). To date, many resistance genes, including *Rlm1-13*, *RlmS*, *LepR1-4*, and *BLMR1-2*, have been identified in *Brassica* species (Ansan-Melayah et al., 1998; Balesdent et al., 2001, 2002; Chevre et al., 2008; Christianson et al., 2006; Delourme et al., 2004; Raman et al., 2013, 2021; Van dee Wouw et al., 2009; Yu et al., 2013). Likewise, 14 avirulence genes have been detected in *L. maculans*, of which eight genes have been cloned: *AvrLm1*, *AvrLm2*, *AvrLm3*, *AvrLm4*, *AvrLmJ1*, *AvrLm6*, *AvrLm7*, and *AvrLm5* (Ghanbarnia et al., 2015, 2018; Plissonneau et al., 2016). As use of resistant resources is the primary management strategy for *L. maculans*, blackleg genetic research has moved towards detecting both *R* and *Avr* genes in the plant and pathogen, respectively (Balesdent et al., 2002, 2005).

Unfortunately, data describing *Avr* genes in Iranian isolates are restricted to determining PGs. Using a cotyledon inoculation test (Williams et al., 1980), *L. maculans* isolates were categorized into four PGs: PG1 to PG4 (Koch et al., 1991), or six PGs: A1 to A6 (Badawy et al., 1991; Kuswinanti et al., 1995). These PGs could be considered as different races of the pathogen, with each race differing in their *Avr* gene content.

The first PGs of *L. maculans* (PG2 and PGT), identified using cotyledon inoculation assays on the differential set of Westar (no *R* genes, susceptible control), Quinta (*Rlm1*, *Rlm3*), and Glacier (*Rlm2*, *Rlm3*), were recorded in the Mazandaran province in the north of Iran, in 2009 (Zamanmirabadi, Rahnama, Esmaeilifar, 2009; Zamanmirabadi, Rahnama, Mehdi Alamdarlou, et al., 2009). Eight years after the first report of PG2 and PGT, PG3 and PGT groups were detected in Golestan province (Vakili-Zarj et al., 2017a).

4.6 | Variety testing and resistance ratings

The first experimental design to investigate the susceptibility of canola cultivars in Iran was undertaken using 32 Australian cultivars and lines (Pacific Seeds Co.; Mehdi Alamdarlou, Zamanmirabadi, Fatmi Naghadeh, 2007). A subsequent cotyledon assay test of three cultivars, Hyola 401 (no *R* genes), Okapi (no *R* genes) and Zarfam, to a PGT isolate (D04-12) showed all cultivars were highly susceptible (Zamanmirabadi, Rahnama, et al., 2010). In another study, the resistance of canola cultivars Hyola 401, Hyola 308, Talaye, Zarfam, Sarigol, Fornax, Modena, Okapi, Option 500, and RGS 003 to one blackleg isolate was investigated under controlled conditions. The cultivars Sarigol and Hyola 401, with disease severities of 19.4% and 17.5%, respectively, were the most susceptible, whilst Fornax and

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Talaye, with disease severities of 5.9% and 5.8%, respectively, were the most resistant cultivars (Banaii et al., 2009). However, there was no information on which PG of *L. maculans* was used for testing. In a further test (Rahmanpour & Alizadeh, 2015), the expanded leaves of 25 lines of 3-month-old canola plants were inoculated and the diameter of induced lesions was measured. Based on the results, three lines (HW101, HW104, and SW104) were resistant. In the latest comprehensive study on the PG groups of 172 *Leptosphaeria* isolates in Mazandaran, Golestan, Ardabil, Khuzestan, and Ilam provinces, a frequency of 51.7% of isolates were PG4, 20.3% were PG1, 16.9% were PG3, 6.4% were PGT, and 4.7% were PG2 (Afshari-Azad et al., 2019).

Due to the restricted availability of a differential set of plant materials harbouring *Rlm* genes, studies on the *Avr* genes present in Iranian isolates are in the early stages (Vakili-Zarj et al., 2017c). The differential reactions of 22 lines and commercial cultivars consisting of 15 *B. napus*, four *B. rapa*, two *B. juncea*, and one *B. nigra* to PG2 and PGT have been examined (Keypoor et al., 2015). Among local cultivars, Option and PF (Sari Gol) showed more resistance and partial resistance to PG2, respectively. Among foreign cultivars, Adriana (*Rlm4*) and Champlain grouped in resistance and partial resistance, respectively, and Option, Adriana and Champlain had a partial resistance to PGT compared to other genotypes (Keypoor et al., 2015). The effects of an increase in temperature on resistance stability to *L. maculans* have been examined by Amjadi et al. (2018), who demonstrated that the resistant (Excel, *Rlm7*) and susceptible (Drakkar, no *Rlm*) cultivars reacted to different temperatures in different ways. In the susceptible cultivar, lesion size became two-times smaller at high temperature (28/23°C) than at the optimum temperature (21/16°C). In contrast, lesion size of the resistant cultivar on cotyledons increased three-fold at high temperatures compared to the optimum temperature. As an increase in lesion size due to higher temperature has been previously shown in Quinta (*Rlm1*) (Badawy et al., 1992) and Darmor MX (*Rlm6*) cultivars (Huang et al., 2006) and reduction in the function of *Rlm1*, *Rlm6*, and *Rlm7* resistance genes have been previously documented, temperature sensitivity is likely to present in other resistance genes.

5 | DISEASE MANAGEMENT

Globally, many strategies have been used to manage blackleg disease, for example, agronomic practices including crop rotation, stubble management (Barbetti & Khangura, 1999; Marcroft et al., 2004), deep tillage, changing sowing date, and nitrogen management (Aubertot et al., 2004; Guo et al., 2005; Khangura & Barbetti, 2004), application of chemicals (Eckert et al., 2010; Huang et al., 2011; Sewell et al., 2016), and planting resistant varieties (Aubertot et al., 2006; Rouxel et al., 2003).

5.1 | Cultural control

There are always many recommendations in observing the rotation of rapeseed with cereals and managing rapeseed residues to reduce the damage of rapeseed disease in Iran. However, due to the small size of cultivated area (on average about 1 ha) and lack of alternative crops to put in rotation and inefficient management of canola residues, it is unfortunately not possible to correctly implement crop management. Although pilot projects are underway to introduce winter crops such as flax and safflower in rotation with canola, they have not yet been widely implemented (Zamanmirabadi et al., 2018b).

5.2 | Races of *L. maculans*

Due to the frequency of their occurrence, there is no specific geographical distribution of *L. maculans* and *L. biglobosa* species in Iran, and during the last decade the pathogen race structure has significantly changed (Vakili-Zarj et al., 2017b; Zamanmirabadi, Rahnama, Esmaeilifar, 2009; Zamanmirabadi, Rahnama, Mehdi Alamdarlou, et al., 2009). In order to make informed decisions on disease management, it is important to know what races are present and in which areas they are most likely to be encountered. Therefore, it is recommended to create a map of the *Leptosphaeria* spp. population distribution in each province, particularly the northern provinces.

5.3 | Disease progress

High rainfall and relative humidity lead to the creation of favourable conditions for the spread of blackleg disease (Ghanbarnia et al., 2009; Lob et al., 2013). During the past 2 years in Iran, dramatic environmental and climate conditions have been observed (Tavousi, 2018). For example, wet periods after drought years have occurred (Fatemi et al., 2015). This situation may lead to blackleg disease incidence and severity and decrease ascospore maturity and release time (Salam et al., 2007). The main cultivation area of canola in Iran is situated in Mazandaran and Golestan provinces, where similar climate conditions and agricultural practices are anticipated, and where more than 90% incidence of blackleg disease is regularly recorded. Thus, effective disease management strategies should take into account changes in climatic conditions in these two provinces.

5.4 | Resistant varieties

The most durable blackleg management strategy is deploying resistant cultivars (REX Consortium, 2016). In two separate experiments, performed in Mazandaran and Golestan provinces, the blackleg incidence and severity were determined in commercial canola cultivars. In Mazandaran, the cultivars PR46H75 and RGS showed the lowest and highest disease severity and incidence, respectively (Abbaspour, 2020). In Golestan, Hyola 76 and Macro were found to have the maximum and minimum tolerance to blackleg, respectively (Zanganeh, 2020). Hyola 76 is a moderately resistant hybrid cultivar in Iran; however, it has been known as

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moderately susceptible in other countries (Kulczynski et al., 2014), suggesting there is a difference in the *L. maculans*/*L. biglobosa* populations in different regions. Thus, resistant cultivars can be used in disease management strategies, as these cultivars could prevent severe population change over time (Van dee Wouw et al., 2014).

A detailed understanding of epidemiological information could help in the development of disease management strategies, including determining the optimum time for fungicide application and adjusting the planting date. As previous investigations on the *Leptosphaeria* spp. populations have confirmed that *L. biglobosa* is the dominant species in the north of Iran (Zamanmirabadi, Rahnama, et al., 2010), less attention has been paid to epidemiological studies of the *L. maculans* population.

5.5 | Chemical control

According to studies in the Mazandaran province, the fungus releases ascospores from late September to early December (Abbaspour, 2020), depending on air temperature (Toscano-Underwood et al., 2003) and rainfall during the summer (Gladders & Symonds, 1995). Although stubble management during the summer season can significantly decrease the risks of infection by ascospores released from canola residue, it is almost impossible to follow this strategy as more than 90% of canola in the north of Iran is sown immediately after the first rainfall, which mainly occurs in the second half of October or by the end of November. Therefore, the application of chemicals is the most effective method to control the disease (Gladders et al., 1998).

The first experimental material for chemical control of blackleg in Iran was nanosilver (silver nanoparticles of 1–100 nm in size). The test was carried out in agar media by applying eight doses (5,10, 30, 50, 100, 120, 130, and 150 ppm) of this material and showed that all concentrations had a prohibition effect on mycelial growth and production of pycnidium 2, 5, 7, 9, and 13 days after inoculation, while doses >100 ppm resulted in complete inhibition of growth. There was no significant difference for doses more than 100 ppm (Zamanmirabadi, Mehdi-Alamdarlou, Esmailifar, 2010).

The only field experiment investigating the effect of chemical treatments (Iprodione + Carbendazim [Rovral T-S], Carbendazim [Bavistin], Tebuconazole [Folicur], Propiconazole [Tilt], and Captan) on blackleg disease showed that the lowest disease intensity (6.7%) was related to seed treatment with Captan, along with Propiconazole foliar application (Dalili et al., 2015).

5.6 | Biological and other treatments

Several biocontrol agents have been shown to be successful for the control of *L. maculans*. The antagonist effect of 15 *Trichoderma* and 17 *B. subtilis* isolates against blackleg infection was studied using canola seeds and plant above-ground parts in the greenhouse. Some *B. subtilis* and *Trichoderma* species isolates decreased the blackleg disease severity in a seed treatment assay. However, only two *Trichoderma* isolates decreased disease severity (up to 60%) when treating above-ground parts (Panjehke et al., 2011).

The antimicrobial effect of 16 medicinal plant extracts on the growth of *Plenodomus lingam* mycelium was studied (Shafiei et al., 2013). It was shown that cinnamon (*Cinnamomum verum*), eucalyptus (*Eucalyptus globulus*), onion (*Allium cepa*), and rosemary (*Rosmarinus officinalis*) extracts reduced *Plenodomus* growth by 47.8%, 39.1%, 22.6%, and 18.2%, respectively; however, under greenhouse conditions, only cinnamon and rosemary essential oils displayed better antifungal efficiency than other extracts (Shafiei et al., 2013).

The application of different treatments, including Iprodin + Carbendazim (Rovral TS 52.5 WP) fungicide, *Trichoderma harzianum*, and *Talaromyces flavus* was tested on commercial cultivars, including Hyola 401 and Hyola 50. The results showed a mixture of application of *T. flavus* (at sowing) and Rovral TS (at flowering) was the best treatment for controlling blackleg under field conditions (Afshari-Azad, Keihanian, et al., 2016). In a further study, the effect of *T. harzianum* and *T. atroviride* against *P. lingam* was investigated in both the laboratory and greenhouse (Akbari et al., 2018). The antagonistic effects on the isolates were tested by treating rapeseed seeds and rapeseed above-ground parts. In dual culture, the two isolates *T. harzianum* and *T. atroviride* could inhibit the growth of the pathogen by 79.2% and 80.2%, respectively. The growth inhibition was 15%–21% and 31%–39% at 120 and 240 h after culture in the volatile compound phase. In greenhouse tests, antagonistic isolates could significantly ($p < 0.01$) decrease disease severity compared to the control treatment. The results also revealed that in the seed treatment experiment, *T. harzianum* and *T. atroviride* isolates reduced disease severity up to 42.5% and 47.5%, respectively, after 2 weeks (Akbari et al., 2018). In the same survey, canola seed treatment with *T. harzianum* isolates T39 and T20 could reduce disease severity by 65.2% and 58.6%, respectively (Ghasemi Alitapeh et al., 2018).

Application of genetic engineering methods, along with biological treatments, can be an alternative to chemical control. For example, introduction of the pea defensin gene *DRR206* in transgenic canola lines increases resistance to *L. maculans* through decreased hyphal growth at inoculation sites (Wang et al., 1999). Expression of *Cf9* and *Avr9* genes in canola transgenic lines has also been shown to increase blackleg resistance (Hennin et al., 2001). Furthermore, expressing an antimicrobial peptide MiAMP1, originally isolated from the seeds of *Macadamia integrifolia*, in transgenic canola has been shown to be a method for management of blackleg disease (Kazan et al., 2002). In addition, introduction of the *CHIT42* gene (containing a chitin-binding domain) from *T. atroviride* for resistance against *Alternaria solani*, into *B. napus* showed that transgenic plants had more resistance than the control plant against fungi (Ziaei et al., 2014).

L. biglobosa is generally less damaging (Fitt et al., 2008; West, Balesdent, et al., 2002) and can activate local and systemic defence responses in canola to decrease *L. maculans* (Mahuku et al., 1996). In Iran, *L. biglobosa* is often associated with less damaging upper stem lesions, although some references confirm *L. biglobosa* can be an important

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cause of blackleg on oilseed rape (Huang et al., 2014; Zamanmirabadi, Mehdi-Alamdarlou, Esmaeilifar, Fathi, 2010). Furthermore, reduction in blackleg severity has been observed when canola plants were treated with ascospores of *L. biglobosa* (Liu et al., 2006). In studies conducted in different parts of Iran (A. Zamanmirabadi, unpublished data), the *L. biglobosa* population was found to be significantly greater than *L. maculans*. Thus, it is recommended that triazole fungicides are not used to control blackleg disease in areas where *L. biglobosa* species is more dominant. However, it has also been observed that *L. biglobosa* isolates were more resistant to the triazole fungicides than *L. maculans* isolates (Eckert et al., 2010).

5.7 | Genetic analysis

The first molecular markers used in Iran for differentiating avirulent and virulent groups of blackleg were RAPD PCR (R2 and R4 primers) and rep-PCR (ERIC, BOX and REP) markers (Palizi et al., 2008). Further molecular research for determining haplotypes was performed using a series of random primers, including OPRI1, OPRI4, OPRI5, OPB11, OPR20, OPA3, and OPA2. However, no specific correlation was observed between geographical regions and haplotypes, although there were different haplotypes in each location (Momeni & Afshari-Azad, 2010).

The sequence analysis of internal transcribed spacer regions 1 and 2 and 5.8S nrDNA (ITS) of 72 Iranian isolates revealed that the sequences were highly conserved (98%–100% identity along a 334 bp alignment) with the identical sequences of known *L. maculans* isolates in GenBank (Vakili-Zarj et al., 2017a). Furthermore, whole-genome sequencing of 95 Iranian isolates of the *Leptosphaeria* spp. population in the north of Iran showed that more than 95% of isolates belong to *L. biglobosa* (A. Zamanmirabadi, unpublished data).

6 | FUTURE PRIORITIES

This review provides comprehensive information on canola cultivation and blackleg disease in Iran, especially its northern regions. Given the short history of *L. maculans* in Iran (Fernando et al., 2007; Zamanmirabadi et al., 2008), little is known about this pathogen. However, considering the government's policy for developing canola cultivation and current reports on the population changes of *L. maculans* during the past decade (Vakili-Zarj et al., 2017a), it is essential to conduct more research on this pathogen. During the last 3 years, the climate has been cooler and the precipitation rate higher in Iran, particularly in the Caspian Sea coastline. This climatic variation will be favourable for *L. maculans*. Therefore, northern regions of Iran, where favourable temperature prevails for fungi, are at a higher risk of pathogen spread. Thus, to develop durable management strategies to control *L. maculans*, we need to understand disease epidemiology mechanisms based on moisture factors (relative humidity and rainfall) and temperature-based models during the summer and autumn seasons.

In Iran's northern regions, farmers intensively perform rice cultivation during high rainfall years after harvesting canola. Planting rice under flooding conditions often reduces the primary inoculation and survival time of *L. maculans* for the next growing season. This growing pattern could be a strategy and become part of rotation plans, potentially suppressing white stem rot and blackleg disease and providing a significant economic benefit to farmers.

The first step in blackleg disease management is preparing a geographic map showing the distribution of aggressive and nonaggressive races in an area. Based on the literature, both *L. maculans* and *L. biglobosa* coexist in many areas worldwide (Fitt et al., 2006). In Iran, like Poland (West, Jedryczka, et al., 2002), China (Zhang et al., 2014), and Lithuania (Brazauskiene et al., 2011), *L. biglobosa* is the dominant species and causes minor damage. The primary critical factor for blackleg disease management is to enhance the knowledge on the rate of aggressive races incidence.

Although there is a high similarity between Iranian and global isolates in terms of morphology, host range, and physiological characteristics, there is no information about phylogenetic relationships among the isolates. Therefore, understanding the genetic relationships between Iranian and global isolates should be the priority in future studies (Vincenot et al., 2008; Voigt et al., 2005).

Advanced molecular techniques such as whole-genome sequencing and whole-genome mapping lead us towards identifying a broad spectrum of genetic variation throughout the genome of microorganisms like *L. maculans*. Unfortunately, in Iran, there is no evidence of using this technology to discover *Avr* and *Rlm* genes in the pathogen and host, respectively.

Overall, application of molecular techniques and tools like next-generation sequencing, *Avr* gene monitoring, identification of the predominant population of fungus in the north of Iran as the main centre of disease, quarantine of infected imported seeds, detection and deployment of resistance resources containing *Rlm* genes, forecasting disease using modelling tools under variable climate conditions, determining the role of weeds in the transformation of disease, continuous assessment of the sensitivity of imported hybrids to canola blackleg, evaluation of the efficiency of new fungicides, and finally the possibility of biological control due to the satisfactory results of some biocontrol agents such as *Trichoderma* spp. and *T. flavus* (Afshari-Azad, Dalili, et al., 2016; Akbari et al., 2018; Panjehkeh et al., 2011) should be introduced as part of a most critical research programme for disease management of canola blackleg in Iran.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable as no new data were created or analysed.

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