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New or Unusual Disease Reports

First report of *Leptosphaeria maculans* and *Leptosphaeria biglobosa* causing blackleg disease of oilseed rape in Tunisia

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Summary. Blackleg has been observed in oilseed rape in Tunisia since 2017. Morphological observations, pathogenicity tests, and sequencing of the internal transcribed spacer regions for four fungal isolates from affected plants confirmed the presence of *Leptosphaeria maculans* and *Leptosphaeria biglobosa*. These results provide the first record of *L. maculans* and *L. biglobosa* as causes of blackleg of oilseed rape in Tunisia.

Keywords. Blackleg disease, oilseed rape, fungal identification.

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INTRODUCTION

Brassica napus L. is one of the most common domesticated *Brassica* crops for human and animal nutrition (Friedt *et al*., 2018). Oilseed rape is estimated to occupy more than 35.6 million hectares (ha) of world agricultural area with average production of 71 million tons (t) in the 2021–2022 growing season (World Agricultural Production, 2022). The oilseed rape crops have been reintroduced into the Tunisian national cultivation systems since 2014 (Medimagh *et al*., 2018), and average yields have increased from 1.3 t ha^{-1} in 2014–2015 to 1.8 t ha^{-1} in 2018-2019 (Maghreb Oléagineux, 2022). With more than 15,000 ha of current rapeseed crop area, these crops are important in Tunisia, especially in the northern regions of the country (Maghreb Oléagineux, 2022).

Five hybrid European spring varieties are currently subscribed in the Tunisian catalogue of varieties and dominate *Brassica napus* cultivation in Tunisia (Maghreb Oléagineux, 2022). Reductions in oilseed rape yields have

been noticed due to biotic stress (Wang *et al*., 2020; Zheng *et al*., 2020). Blackleg (Phoma stem canker) is an important fungal disease threat to international oilseed rape production (Howlett, 2004; Fitt *et al*., 2006). *Leptosphaeria maculans* (Desm.) Ces. and de Not. (anamorph *Phoma lingam*) is the principal cause of this disease together with *L. biglobosa* Shoemaker and H. Brun (*L. biglobosa*) (Rouxel *et al*., 1994; Shoemaker and Brun, 2001). *Leptosphaeria biglobosa* is considered to be less aggressive than *L. maculans*, and often attacks the upper parts of host plants (Williams, 1999; Shoemaker and Brun, 2001; Mendes-Pereira *et al*., 2003). In 2017, symptoms similar to blackleg were observed in Beja, Bizerte, Nabeul and Manouba, the four main oilseed rape production areas of Tunisia.

The objective of the present study was to identify the causal agents responsible for the blackleg on oilseed rape in Tunisia. Cultural and morphological features, molecular sequencing of the internal transcribed spacer (ITS) region, phylogenetic analysis, and pathogenicity tests were performed for isolates of fungi obtained from oilseed rape crops.

MATERIALS AND METHODS

Isolation and morphological identification of causal agents

To isolate the causal agent, diseased oilseed rape plants were sampled from four northern regions of Tunisia (nine fields) during April and May 2018. The samples were conveyed to the Pests and Integrated Protection in Agriculture research laboratory in Tunisia. For each sample, five infected stem sections (5 cm length) were surface-sterilized in 1% sodium hypochlorite solution for 30 s, followed by 70% ethanol for 20 s, and three rinses in sterile water. The stem sections were then transferred into 90 mm diam. Petri dishes containing V8 juice agar supplemented with 25 mg mL^{-1} of chloramphenicol. After 15 d incubation under 12 h photoperiod at 20°C, serial dilutions were performed to obtain single conidium isolates that were then maintained on V8 juice agar at 20°C. From the initial isolate collection (Table 1), four isolates (obtained from four fields) were randomly selected for morphological and genetic identifications. Isolates L31 and L36 were from two fields in Manouba and isolates L48 and L50 were from two fields in Nabeul.

For macroscopic identification, 5 mm mycelium agar discs of each isolate were inoculated onto 90 mm diam. Petri dishes containing malt agar, V8 juice agar or potato dextrose agar (PDA). Colonies were photographed at 7 and 14 d after incubation at 20°C in complete darkness. For each isolate, colony colour, and conidium size and shape were analyzed under light microscope and then measured using ImageJ software (Schneider *et al*., 2012).

Molecular and phylogenetic analyses of the causal agents

Genomic DNA was extracted from the four selected isolates using the CTAB protocol (Doyle and Doyle, 1987). PCR was performed to amplify the ITS region using the forward ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and the reverse ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primer, as described by White *et al*. (1990). The amplifications were carried out using the Thermo Cycler 2720 (Applied Biosystems) in 25 µL reaction mixtures. PCR conditions were as follows: 4 min of initial denaturation at 94°C, followed by 30 cycles of denaturation each at 94°C for 1.5 min, 2 min of annealing at 55°C and 3 min of extension at 72°C, with a final elongation at 72°C for 10 min. A Sanger sequencing using both directions was carried out by CarthaGenomics Advanced Technologies (Tunis, Tunisia). The consensus ITS sequence of each

Table 1. Details of Tunisian regions from which *Leptosphaeria* spp. isolates were sampled in *Brassica napus* crops in 2018.

Region	Municipality	Commune	Field No.	Latitude	Longitude	Altitude (m)	Number of sampled isolates
Beja	Beja North	Ghyria	1	36.72900	9.135720	409	6
Beja	Beja North	Beja	2	36.72200	9.192360	245	1
Beja	Testour	Oued Zargua	3	36.66700	9.444870	169	2
Bizerte	Tinjah	Tinjah	4	37.17681	9.769790	4	4
Bizerte	Tinjah	Tinjah	5	37.15695	9.765250	15	3
Manouba	Tebourba	Chouigui	6	36.90000	9.850000	67	6 *(Inc. Isolate L36)*
Manouba	Tebourba	Eddekhila	7	36.89000	9.728000	74	5 *(Inc. Isolate L31)*
Nabeul	Menzel Bouzalfa	Menzel Bouzalfa North	8	36.69120	10.59816	58	3 *(Inc. Isolate L48)*
Nabeul	Menzel Bouzalfa	Errahma	9	36.71000	10.74000	133	4 *(Inc. Isolate L50)*

Table 2. Isolates of the *Leptosphaeria maculans* and *Leptosphaeria biglobosa* species complex used as references in the phylogenetic analyses.

Species	Subclade	Isolate name	IBCN number	Origin	Host	GenBank accession number	References
Leptosphaeria maculans	'brassicae'	IRAN Br37	-	Iran	-	MG701143	Amirdehi *et al.*, 2017
		Pk4	-	Iran	*B. napus*	MW444866	Zamanmirabadi *et al.*, 2022
		UK7	-	UK	*B. napus*	DQ133891	Liu *et al.*, 2006
		CBS 275.63	-	UK	-	MW810266	Zhao *et al.*, 2021
		Leroy / FSU428	80	Canada	*B. napus*	AJ550883	Mendes-Pereira *et al.*, 2003; Voigt *et al.*, 2005
	'lepidii'	Lep-2 / FSU432	84	Canada	*Lepidium* sp.	AJ550890	
Leptosphaeria biglobosa	'thlaspii'	92-01-2 / FSU373	65	Canada	*T. arvense*	AJ550891	
		CBS303.51	-	France	*Isatis tinctoria*	AJ550892	
	'australensis'	2.1 / FSU415	29	Australia	*B. napus*	AJ550869	
		PHW1268 / PHW126 / FSU471	91	USA	*B. oleracea*	AJ550870	
	'erysimii'	Ery-2 / FSU431	83	Canada	*Erysimum* sp.	AJ550872	
	'brassicae'	PL53	-	Poland	*B. napus*	AJ550865	
		2379-4 / FSU437	89	Canada	*B. napus*	AJ550863	
		PHW1270 / PHW129 / FSU473	93	USA	*B. oleracea*	AJ550857	
		CBS127249	-	France	*B. juncea*	JF740199	De Gruyter *et al.*, 2012
		HNO96	-	China	*B. napus*	MZ312591	-
		UK5	-	UK	*B. napus*	DQ133890	Liu *et al.*, 2006
	'americensis'	Phl002	-	USA	*B. rapa*	MG321243	Zou *et al.*, 2019
	'occiaustralensis'	UWA21-8	-	Australia	*B. napus*	AM410082	Vincenot *et al.*, 2008
	'canadensis'	Lb1135	-	China	*B. napus*	MK335624	Luo *et al.*, 2021
		BJ-114 / FSU430	82	Canada	*B. juncea*	AJ550866	Mendes-Pereira *et al.*, 2003 Voigt *et al.*, 2005

-: Unknown or not available information

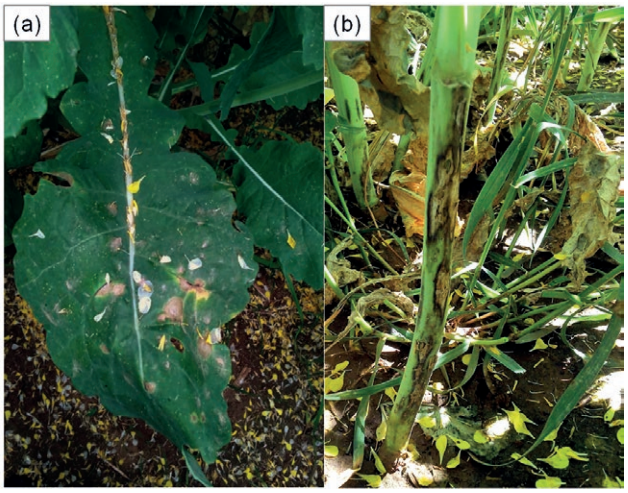

Figure 1. Blackleg (Phoma stem canker) symptoms on oilseed rape plants observed during field sampling in Tunisia in spring 2018. (a) Lesions observed on an upper leaf of the cv. PR45H73, in the Manouba region. (b) Severe symptoms on a stem of the cv. Trapper, in the Bizerte region.

isolate was then analyzed using the Basic Local Alignment Search Tool (BLAST) software on NCBI available from: https://www.ncbi.nlm.nih.gov/. The best match for each sequence with the lowest *E*-value and the highest query cover and percentage of identity was recorded. In addition, a phylogenetic analysis was carried out by aligning, using the ClustalW algorithm, the ITS sequences of the four isolates from Tunisia with available reference sequences of *L. maculans* and *L. biglobosa* in the GenBank database. Isolates from different countries of origin, representative of previously published *Leptosphaeria* subclades, were used in this assessment (Mendes-Pereira *et al.*, 2003, Voigt *et al.*, 2005, Liu *et al.*, 2006, Vincenot *et al.*, 2008, De Gruyter *et al.*, 2012, Amirdehi *et al.*, 2017, Zou *et al.*, 2019, Zhao *et al.*, 2021, Luo *et al.*, 2021, Zamanmirabadi *et al.*, 2022) (Table 2). A phylogenetic tree was obtained using the Maximum Likelihood method and the Tamura-Nei model (Tamura and Nei, 1993) with 1000 bootstrap replications, under the Mega XI software (Tamura *et al.*, 2021).

Pathogenicity tests

Seedlings of oilseed rape cv. Topas (which has no major resistance genes (Larkan *et al*., 2016)) were grown under controlled conditions of 20°C, 90% relative humidity and 16 h light, 8 h dark cycles. Using the cotyledon assay for pathogenicity (Bonman *et al*., 1980), nine plants were slightly wounded in each cotyledon lobe with a sterile needle, and were then each inoculated with a 10 µL spore suspension at 1×10^7 conidia mL^{-1} (Winter and Koopmann, 2016; Alnajar *et al*., 2022). Mock inoculations with only sterile distilled water were also carried out in a similar manner. Each isolate and water controls were inoculated onto nine plants. Symptom evaluations were carried out 14 d post-inoculation using the IMASCORE rating scale (Volke, 1999; Balesdent *et al*., 2001). *Leptosphaeria maculans* that gave sporulating grey-green tissue collapse in inoculated seedlings was re-isolated and morphologically identified, to assess Koch's postulates.

RESULTS AND DISCUSSION

In the northern visited oilseed rape fields of Tunisia, typical symptoms of blackleg were observed on the crop plants, that included large green to grey-coloured leaf spots and basal stem lesions, which were cream to pale brown thick dark brown borders. Lesions on living plants and on 2-month-old crop residues contained multiple pycnidia often releasing pink mucilage. Disease incidence was variable from one sampled field to another, but ranged between 48 and 100%. No severe attack leading to plant lodging was observed in the northern oilseed rape growing regions of Tunisia in 2018.

After 14 d of incubation, the fungal isolates L31, L36, and L50 had regular white-dark green colonies on PDA and white-brown mycelium on V8 agar, and irregular small brown to black colonies on malt agar. Colonies of isolate L48 varied from distinct brownish-yellow with yellow pigmentation on PDA and malt agar

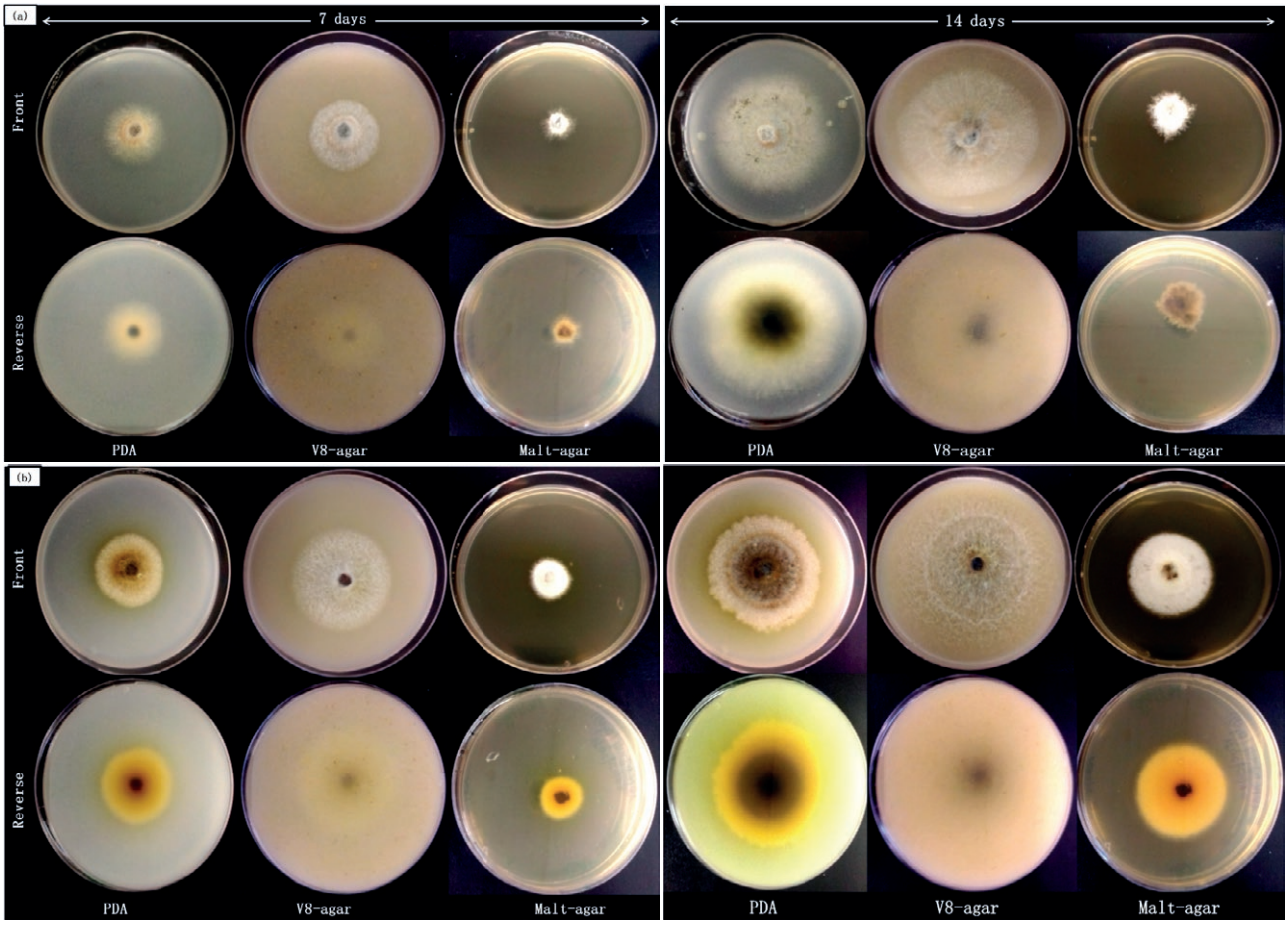

Figure 2. Front and reverse sides of cultures of *Leptosphaeria maculans* isolate L50 (a) and *Lepotosphaeria biglobosa* isolate L48 (b), grown for 7 or 14 d at 20°C in the dark on Potato Dextrose agar (PDA), V8 agar or malt agar.

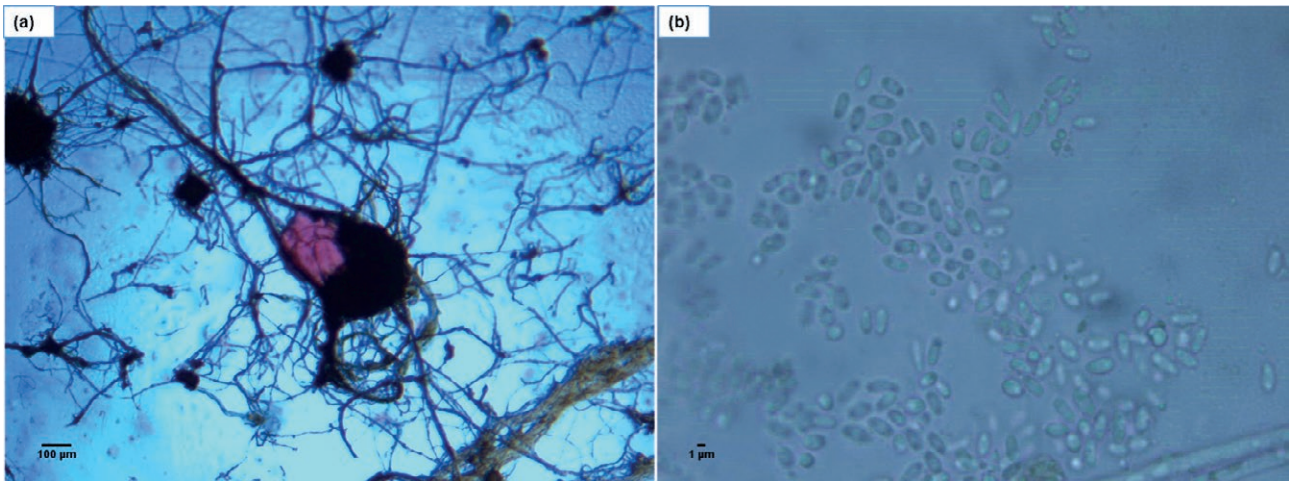

Figure 3. Micrographs of *Lepotosphaeria maculans* isolate L50 after 21 d on V8 agar at 20°C in the dark. (a) Pycnidia with typical pinkish mucilage (40 ×), and (b) pycniospores (conidia) (100 ×).

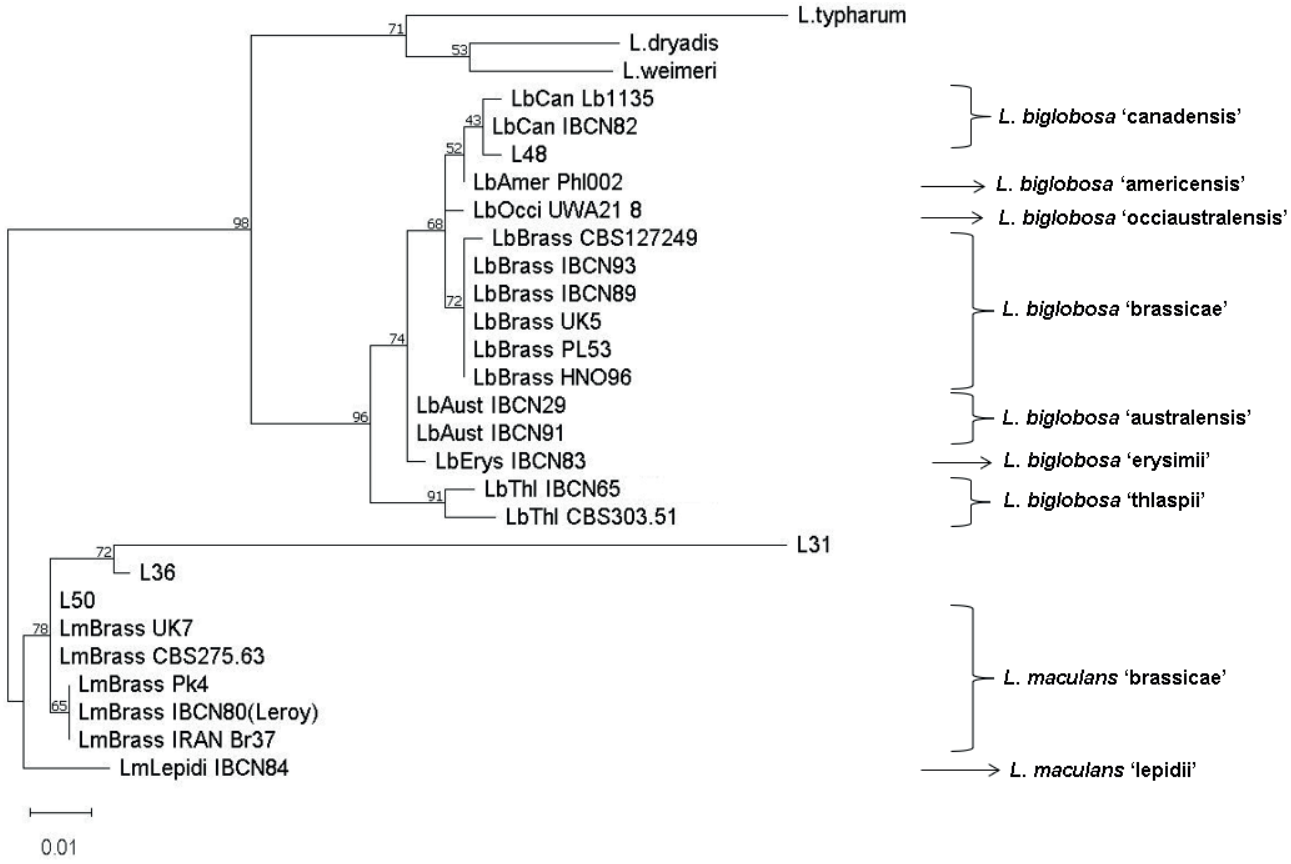

Figure 4. Phylogenetic tree of *Leptosphaeria maculans* and *Leptosphaeria biglobosa* isolates performed on the ITS sequenced region based on the Maximum Likelihood method and Tamura-Nei model. This analysis was carried out using Mega XI software (Tamura *et al.*, 2021), with 1000 bootstrap replications, and involved 28 nucleotide sequences of 327 final nucleotide positions [four sampled isolates from Tunisia, 21 other representative isolates of each subclade available in GenBank, and three different *Leptosphaeria* species (Câmara *et al.*, 2002): *L. typharum* (AF439465), *L. dryadis* (AF439461) and *L. weimeri* (AF439466)].

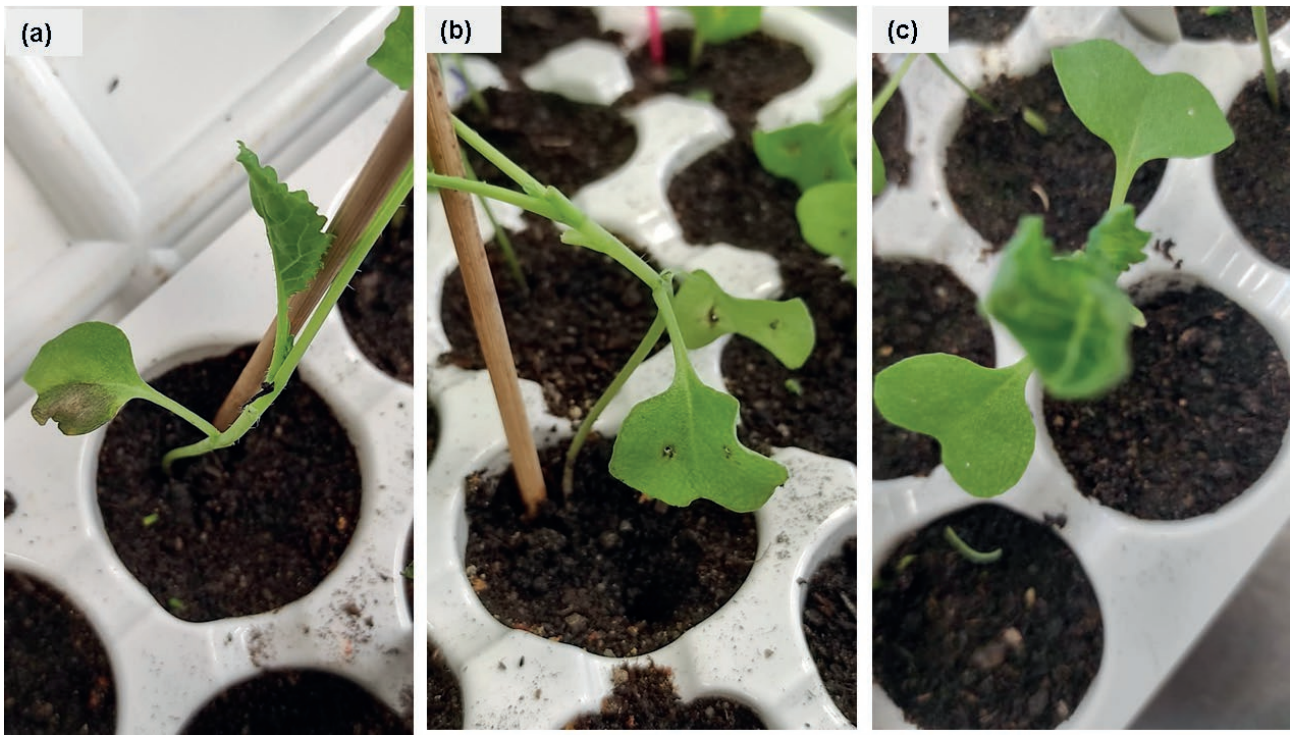

Figure 5. Leaf lesions on 14-d-old seedlings of oilseed rape cv. Topas (Larkan *et al.* 2016) inoculated with *Leptosphaeria maculans* isolate L50 (a) or *Leptosphaeria biglobosa* isolate L48 (b), under controlled conditions (20°C and 16 h dark 8 h light per day). Compatible (leaf spots) reactions were observed for L50 (a), and hypersensitive reactions were observed for L48 (b). No symptoms occurred on control plants inoculated with sterile water (c).

to white and dark-brown with notable aerial mycelium on V8 agar (Figure 2). Similar morphological characteristics have been previously reported, and supported identification of *L. maculans* for isolates L31, L36, and L50; and *L. biglobosa* for isolate L48 (Somda *et al.*, 1996; Howlett *et al.*, 2001; Chen *et al.*, 2010; Vakili Zarj *et al.*, 2017). The pycnidia in cultures were black and globose, and 200–500 µm in diameter. The pycnidia each had an ostiole, from which a conidial cirrhus protruded (Figure 3a). Each cirrhus had a mucilaginous texture and was light pink. No cirrhus colour variations toward bright red or "oxblood", as described by Rouxel *et al.* (1994), were observed for the isolates. All conidia were single-celled, hyaline, ovoid to cylindrical, and of dimensions 2–4 × 1–2 µm (Figure 3b). The shape and size of the observed conidia and pycnidia for all four isolates corresponded to the descriptions for *Leptosphaeria* species (Somda *et al.*, 1996; Howlett *et al.*, 2001).

Sequenced ITS fragments for the four isolates were registered in GenBank under the accession numbers MZ542280 to MZ542283. Molecular analyses confirmed the identification of *L. maculans* and *L. biglobosa* from oilseed rape fields in Tunisia. Blast results of the ITS sequences against the NCBI database showed that isolate L48 had 91% similarity with *L. biglobosa* subgroup 'canadensis' (GenBank number KJ574217). The remaining three isolates showed 86% (for L31), 93% (for L36), and 97% (for L50) similarity with *L. maculans* subgroup 'brassicae' (GenBank number KT225526).

The phylogenetic analyses using the ITS sequences of the four isolates from Tunisia and *L. maculans* reference isolates revealed that *L. maculans* isolates L31, L36 and L50 were most related to the reference isolate IBCN80 (GenBank number AJ550883) belonging to the *L. maculans* 'brassicae' subgroup (Figure 4). The *L. biglobosa* isolate L48 was closely related to all *L. biglobosa* 'canadensis' reference isolates, including IBCN63 (GenBank number AJ550868) and IBCN82 (GenBank number AJ550872).

The pathogenicity tests showed that for the three *L. maculans* isolates, typical host symptoms of grey-green tissue collapse with numerous pycnidia (IMASCORE = 6) were visible at 14 d post-inoculation (Figure 5a). For the *L. biglobosa* isolate L48, an hypersensitive reaction was developed (IMASCORE = 1) (Volke, 1999; Balesdent *et al.*, 2001) for all replicates (Figure 5b). These

phenotypic results were similar to those found by Zou *et al.* (2019), and confirmed the grouping of L48 to the subclade 'canadensis'. To fulfil Koch's postulates, *L. maculans* was re-isolated from the artificially inoculated plants. All control plants had no disease (Figure 5c).

This report is the first of *Leptosphaeria maculans* and *Leptosphaeria biglobosa* on oilseed rape in Tunisia, causing blackleg. *L. maculans* has been previously identified on wild radish (*Raphanus raphanistrum* L.) in Tunisia (Djebali *et al.*, 2009). The information from the present study will be useful for future blackleg diagnosis and disease management in oilseed rape in Tunisia.

REFERENCES

Alnajar D., von Tiedemann A., Koopmann B., 2022. Efficacy of blackleg major resistance genes in *B. napus* in Germany. *Pathogens, Multidisciplinary Digital Publishing Institute* 11: 461. DOI: 10.3390/pathogens11040461.

Amirdehi E., Fotouhifar K.-B., Javan-Nikkhah M., 2017. Morphological and molecular study on some species of *Phoma* and related taxa in Iran. *Rostaniha*, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran 18: 59–76. DOI: 10.22092/botany.2017.113235.

Balesdent M.H., Attard A., Ansan-Melayah D., Delourme R., Renard M., Rouxel T., 2001. Genetic control and host range of avirulence toward *Brassica napus* cultivars Quinta and Jet Neuf in *Leptosphaeria maculans*. *Phytopathology* 91: 70–76. DOI: 10.1094/PHYTO.2001.91.1.70.

Bonman J.M., Gabrielson R.L., Williams P.H., Delwiche P.A., 1980. Virulence of *Phoma lingam* to cabbage. *Proceedings 2nd Southeast Asian Symposium on Plant Diseases in the Tropics*, Bangkok, Thailand, 20-26 October 1980.

Câmara M.P.S., Palm M.E., van Berkum P., O'Neill N.R., 2002. Molecular phylogeny of *Leptosphaeria* and *Phaeosphaeria*. *Mycologia* 94: 630–640. DOI: 10.1080/15572536.2003.11833191.

Chen G.Y., Wu C.P., Li B., Su H., Zhen S.Z., An Y.L., 2010. Detection of *Leptosphaeria maculans* from imported canola seeds. *Journal of Plant Diseases and Protection* 117: 173–176. DOI: 10.1007/BF03356356.

De Gruyter J., Woudenberg J.H.C., Aveskamp M.M., Verkley G.J.M., Groenewald J.Z., Crous P.W., 2012. Redisposition of phoma-like anamorphs in *Pleosporales*. *Studies in Mycology* 75: 1–36. DOI: 10.3114/sim0004.

Djebali N., Scott J., Jourdan M., Souissi T., 2009. Fungi pathogenic on wild radish (*Raphanus raphanistrum* L.) in northern Tunisia as potential biocontrol agents. *Phytopathologia Mediterranea* 48: 205–213.

Doyle J.J., Doyle J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

Fitt B.D.L., Brun H., Barbetti M.J., Rimmer S.R., 2006. World-wide importance of phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) on oilseed rape (*Brassica napus*). *European Journal of Plant Pathology* 114: 3–15. DOI: 10.1007/s10658-005-2233-5.

Friedt W., Tu J., Fu T., 2018. Academic and economic importance of *Brassica napus* rapeseed. In: *The Brassica napus Genome* (S. Liu, R. Snowdon and B. Chalhoub, eds.), Cham, Springer International Publishing, 1–20.

Howlett B.J., Idnurm A., Pedras M.S.C., 2001. *Leptosphaeria maculans*, the causal agent of blackleg disease of Brassicas. *Fungal Genetics and Biology* 33: 1–14. DOI: 10.1006/fgbi.2001.1274.

Howlett B., 2004. Current knowledge of the interaction between *Brassica napus* and *Leptosphaeria maculans*. *Canadian Journal of Plant Pathology* 26: 245–252.

Larkan N.J., Yu F., Lydiate D.J., Rimmer S.R., Borhan M.H., 2016. Single R gene introgression lines for accurate dissection of the *Brassica* - *Leptosphaeria* pathosystem. *Frontiers in Plant Science* 7: 1771. DOI: 10.3389/fpls.2016.01771.

Liu S.Y., Liu Z., Fitt B.D.L., Evans N., Foster S.J., … Lucas J.A., 2006. Resistance to *Leptosphaeria maculans* (phoma stem canker) in *Brassica napus* (oilseed rape) induced by *L. biglobosa* and chemical defence activators in field and controlled environments. *Plant Pathology* 55: 401–412. DOI: 10.1111/j.1365-3059.2006.01354.x.

Luo T., Li G., Yang L., 2021. First report of *Leptosphaeria biglobosa* 'canadensis' causing blackleg on oilseed rape (*Brassica napus*) in China. *Plant Disease*, Scientific Societies 105: 3760. DOI: 10.1094/PDIS-12-20-2735-PDN.

Maghreb Oléagineux, 2022. Présentation du programme Maghreb Oléagineux Tunisie. Available at: http://maghreboleagineux.com/le-programme/. Accessed June 26, 2022.

Medimagh S., Mechri M., Mastouri M., Ben H., Khamassi K., 2018. Genetic variability studies for morphological and qualitative attributes among spring rapeseed (*Brassica napus* L.) varieties under rainfed conditions of Tunisia. In: *Proceedings International Congress on Oil and Protein Crops*. Moldova Academy of Science, Chisinau, Moldova. 20-24 May 2018 41.

Mendes-Pereira E., Balesdent M.-H., Brun H., Rouxel T., 2003. Molecular phylogeny of the *Leptosphaeria macu-*

lans- L. biglobosa species complex. *Mycological Research* 107: 1287–1304. DOI: 10.1017/S0953756203008554.

Rouxel T., Gall C., Balesdent M., 1994. Du polymorphisme au complexe d'espèces : combien d'agents pathogènes sont impliqués dans la nécrose du collet du colza ? *Agronomie* 14: 413–432.

Schneider C.A., Rasband W.S., Eliceiri K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods, Nature Publishing Group* 9: 671–675. DOI: 10.1038/nmeth.2089.

Shoemaker R.A., Brun H., 2001. The teleomorph of the weakly aggressive segregate of *Leptosphaeria maculans*. *Canadian Journal of Botany* 79: 412–419. DOI: 10.1139/b01-019.

Somda I., Renard M., Brun H., 1996. Morphology, pathogenicity and isozyme variation amongst French isolates of *Leptosphaeria maculans* recovered from *Brassica juncea* cv. Picra. *Plant Pathology* 45: 1090–1098. DOI: 10.1046/j.1365-3059.1996.d01-190.x.

Tamura K., Nei M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10:512-526.

Tamura K., Stecher G., Kumar S., 2021. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*. Available at: https://doi.org/10.1093/molbev/msab120.

Vakili Zarj Z., Rahnama K., Nasrollanejad S., Yamchi A., 2017. Morphological and molecular identification of *Leptosphaeria maculans* in canola seeds and flowers collected from the north Iran. *Archives of Phytopathology and Plant Protection* 50: 526–539. DOI: 10.1080/03235408.2017.1339986.

Vincenot L., Balesdent M.H., Li H., Barbetti M.J., Sivasithamparam K., Gout L., Rouxel T., 2008. Occurrence of a new subclade of *Leptosphaeria biglobosa* in western Australia. *Phytopathology* 98: 321–329. DOI: 10.1094/PHYTO-98-3-0321.

Voigt K., Cozijnsen A.J., Kroymann J., Pöggeler S., Howlett B.J., 2005. Phylogenetic relationships between members of the crucifer pathogenic *Leptosphaeria maculans* species complex as shown by mating type (MAT1-2), actin, and β-tubulin sequences. *Molecular Phylogenetics and Evolution* 37: 541–557. DOI: 10.1016/j.ympev.2005.07.006.

Volke, b. 1999. *Leptosphaeria maculans*, der erreger der wurzelhals-und stengelfäule an raps: verbreitung verschiedener pathogenitäts-gruppen in europa, quantifizierung des befalls und shadwirkung imfreiland. Ph.D. thesis, University of Göttingen, Germany.

Wang Y., Strelkov S.E., Hwang S.-F., 2020. Yield losses in canola in response to blackleg disease. *Canadian Journal of Plant Science* 100: 488–494. DOI: 10.1139/cjps-2019-0259.

White T.J., Bruns T., Lee S., Taylor J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols* (M.A. Innis, D.H. Gelfand and J.J.S.J. White, eds.), Academic Press, San Diego, United States of America, 315–322.

Williams, Fitt, 1999. Differentiating A and B groups of *Leptosphaeria maculans*, causal agent of stem canker (blackleg) of oilseed rape. *Plant Pathology* 48: 161–175. DOI: 10.1046/j.1365-3059.1999.00333.x.

Winter M., Koopmann B., 2016. Race spectra of *Leptosphaeria maculans*, the causal agent of blackleg disease of oilseed rape, in different geographic regions in northern Germany. *European Journal of Plant Pathology* 145: 629–641. DOI: 10.1007/s10658-016-0932-8.

World Agricultural Production, 2022, Rapeseed production by country- World Agricultural Production 2021/2022. Available at: https://www.worldagricultural-production.com/crops/rapeseed.aspx, Accessed June 26, 2022.

Zamanmirabadi A., Hemmati R., Dolatabadian A., Batley J., 2022. Genetic structure and phylogenetic relationships of *Leptosphaeria maculans* and *L. biglobosa* in northern regions of Iran. *Archives of Phytopathology and Plant Protection*, Taylor & Francis 55: 1062–1081. DOI: 10.1080/03235408.2022.2081653.

Zhao P., Crous P.W., Hou L.W., Duan W.J., Cai L., Liu F., 2021. Fungi of quarantine concern for China I: Dothideomycetes. *Persoonia - Molecular Phylogeny and Evolution of Fungi* 47: 45–105. DOI: 10.3767/persoonia.2021.47.02.

Zheng X., Koopmann B., Ulber B., von Tiedemann A., 2020. A global survey on diseases and pests in oilseed rape: Current challenges and innovative strategies of control. *Frontiers in Agronomy* 2: 2:590908. DOI: 10.3389/fagro.2020.590908.

Zou Z., Zhang X., Parks P., du Toit L.J., Van de Wouw A.P., Fernando W.G.D., 2019. A new subclade of *Leptosphaeria biglobosa* identified from *Brassica rapa*. *International Journal of Molecular Sciences* 20: 1668. DOI: 10.3390/ijms20071668.