A study on *Leptosphaeria maculans* populations in Germany calls for more cautious deployment of the major R genes

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Background

Leptosphaeria maculans, the causal agent of blackleg disease, can cause economically significant yield losses in oilseed rape (OSR). Using resistant varieties has been considered as one of the most effective control methods. Two types of resistance are available in OSR, major gene resistance and quantitative resistance. Due to the fact that major (R) gene resistance is race-specific and *L. maculans* (LM) is a pathogen of high evolutionary potential, monitoring newly evolved LM races is fundamental to detect changes of efficacy of commercially deployed major R genes and thus, providing farmers with reliable recommendation regarding the use of OSR cultivars with efficient resistance. This requires to determine the race structure of LM populations which has been conducted in four regions (Fig. 1) in Germany.

Methods

LM isolates obtained from NK Bravour were phenotyped by inoculating cotyledons of oilseed rape differential lines bearing *Rlm1, Rlm2, Rlm3, Rlm4, Rlm7, Rlm9, LepR1, LepR2* and *LepR3* genes. Monitoring was performed 14 dpi based on the IMASCORE rating scale (Fig. 2). In addition, genotypic characterization was performed using gene-specific PCR assays to define the presence of *Avrlm6* and *Avrlm11* (Tab. 1).

Tab. 1 Primer sequences used to characterize *L. maculans isolates*

Locus	Primer sequences	References
AvrLm6	CGGTAGACGTGATGGAGTTGAC	Fudal *et al.* 2009
	TTAAGATTAGGCGAGAAGCAAGTG	Fudal *et al.* 2009
AvrLm11	TGCGTTTCTTGCTTCCTATATTT	Balesdent *et al.* 2013
	CAAGTTGGATCTTTCTCATTCG	Balesdent *et al.* 2013

Fig. 1 Regions in Germany where *Leptosphaeria maculans* populations were investigated

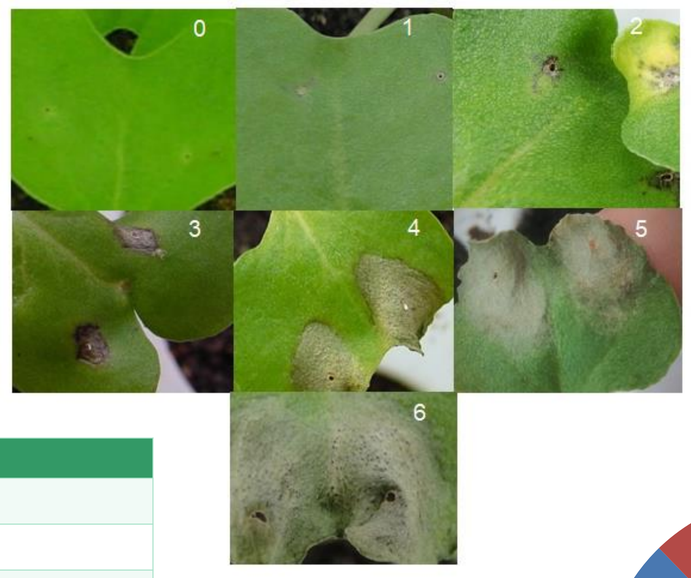

Fig. 2 IMASCORE rating scale to characterize *Leptosphaeria maculans after applying cotyledon test*

Tab. 2 Frequency of avirulence complexity of *L. maculans* isolates in four regions in Germany. Results reflect functioning *Avr* alleles based on phenoytpic assessment.

Avirulence complexity of *L. maculans*	Frequency %
Avrlm7, Avrlep1	23.7
Avrlm3, AvrlepR1	3.2
Avrlm7, AvrlepR1, AvrlepR2	32.7
Avrlm7, AvrlepR1, AvrlepR2, AvrlepR3	8.3
Avrlm7, AvrlepR1, AvrlepR3	4.5
Avrlm1, Avrlm7, AvrlepR1, AvrlepR2, AvrlepR3	5.1
Avrlm1, Avrlm7, AvrlepR1, AvrlepR3	4.5
Avrlm4, Avrlm7, AvrlepR1, AvrlepR2	1.3
Avrlm3, Avrlep1, Avrlep2	3.2
Avrlm7, Avrlep1, AvrlepR2, AvrlepR3	1.3
Avrlm7, AvrlepR1, AvrlepR3	0.6
Avrlm1, Avrlm4, Avrlm7, AvrlepR3	0.6
Avrlm1, Avrlm4, Avrlm7, AvrlepR1, AvrlepR3	0.6
Avrlm1, Avrlm4, Avrlm7, AvrlepR1, AvrlepR2	0.6
Avrlm1, Avrlm7, AvrlepR1, AvrlepR2, AvrlepR3	1.3
Avrlm4, Avrlm7, Avrlep1, AvrlepR3	0.6
Avrlm3, AvrlepR1, AvrlepR3	0.6
Avrlm1, Avrlm7, AvrlepR2, AvrlepR3	0.6
Avrlm1, Avrlem7, AvrlepR1, AvrlepR3	0.6
Avrlm1, Avrlm3, AvrlepR1, AvrlepR2, AvrlepR3	0.6
Avrlm3, AvrlepR1, AvrlepR2, AvrlepR3	0.6
Avrlm1, Avrlm3, AvrlepR1, AvrlepR3	0.6

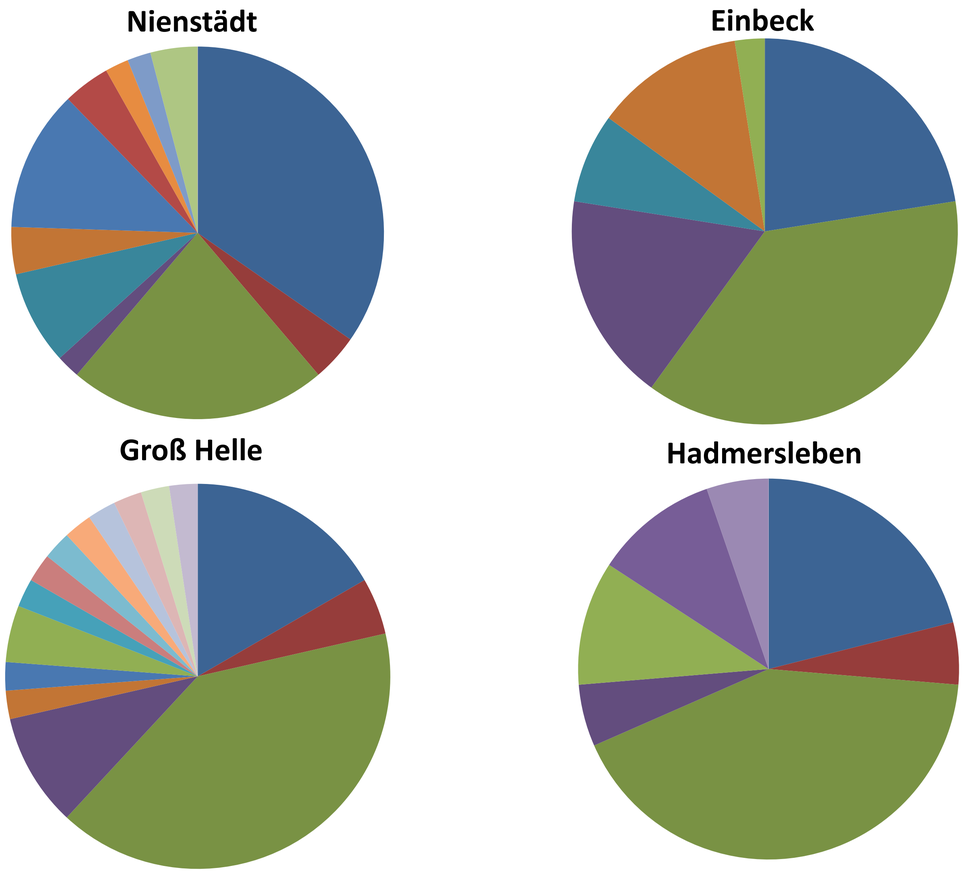

Fig. 4 Frequency of avirulence complexity of *L. maculans* isolates in different regions. For colour coding see table 2.

Results

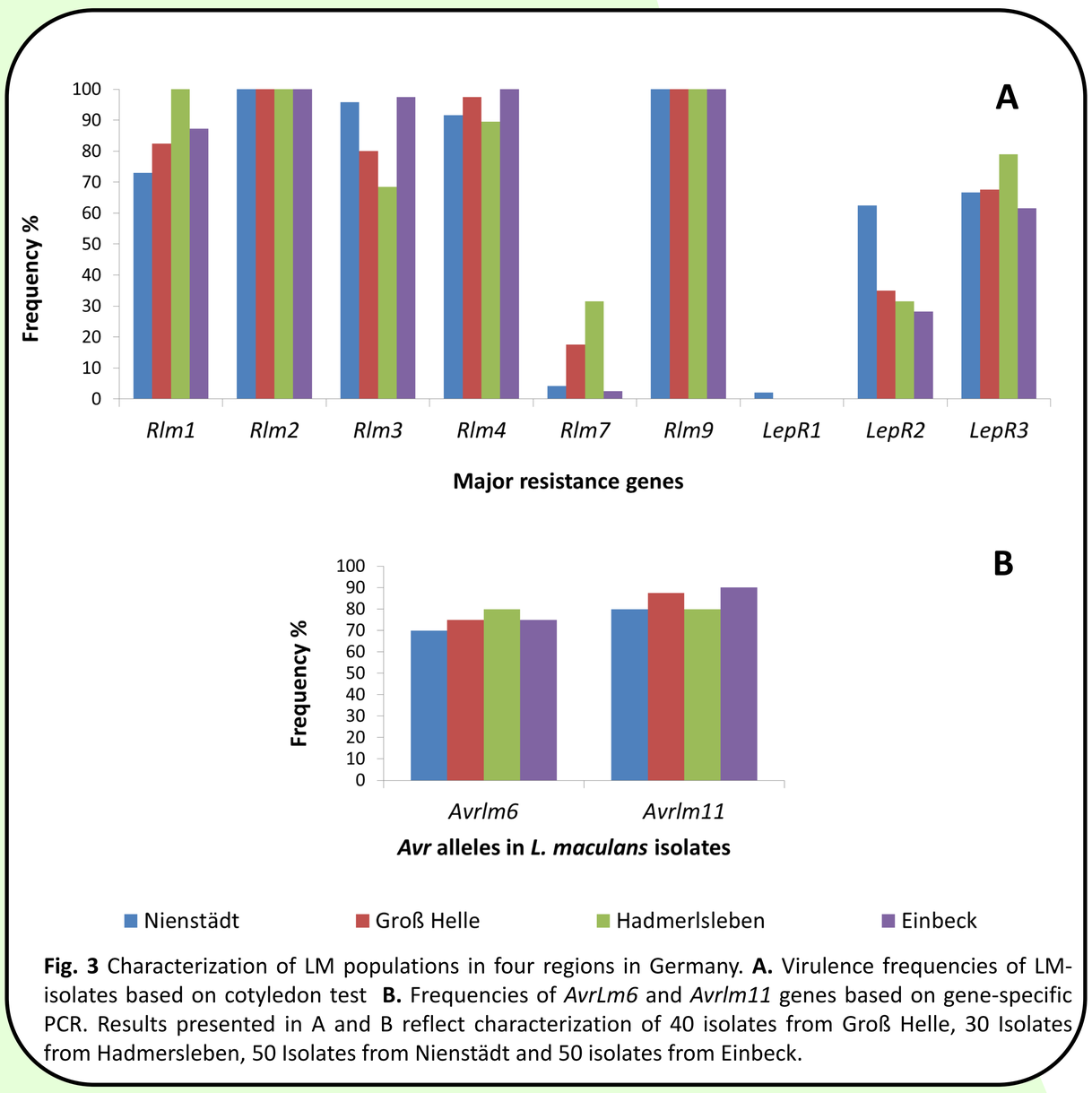

Fig. 3 Characterization of LM populations in four regions in Germany. **A.** Virulence frequencies of LM-isolates based on cotyledon test **B.** Frequencies of *AvrLm6* and *Avrlm11* genes based on gene-specific PCR. Results presented in A and B reflect characterization of 40 isolates from Groß Helle, 30 Isolates from Hadmersleben, 50 Isolates from Nienstädt and 50 isolates from Einbeck.

Summary and conclusions

- Low efficacy of *Rlm1, Rlm3* and *Rlm4* was proved in all regions, while the resistance of *Rlm2* and *Rlm9* was 100% broken.
- Phenotyping of low frequencies of *Avrlm3* and *Avrlm9* might be a result of epistatsis effects of *Avrlm4-7* and thereby not precisely displaying low gene frequencies.
- At present, *Rlm7* and *LepR1* represent the most effective major R genes in the studied regions. However, significant decrease of *Rlm7* efficacy was demonstrated in Hadmersleben and Groß Helle (ca. 20%). Therefore, it is necessary to increase the awareness of the risk related to use one major R-genes over seasons.
- Sharing information about the commercially deployed major R-genes in the German market between cultivar producers and scientists is necessary to provide the farmers with concrete strategies to rotate the use of major R-genes and keep their efficacy.

Literature:

- Fudal, Isabelle; Ross, Simon; Brun, Hortense; Besnard, Anne-Laure; Ermel, Magali; Kuhn, Marie-Line et al. (2009): Repeat-induced point mutation (RIP) as an alternative mechanism of evolution toward virulence in Leptosphaeria maculans. In Molecular plant-microbe interactions : MPMI 22 (8), pp. 932–941. DOI: 10.1094/MPMI-22-8-0932.
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