ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 105, No. 3 (2018), p. 265–270
DOI 10.13080/z-a.2018.105.034

Genetic diversity of *Botrytis cinerea* from strawberry in Lithuania

Neringa RASIUKEVIČIŪTĖ, Rytis RUGIENIUS, Jūratė Bronė ŠIKŠNIANIENĖ

Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry
Kauno 30, Babtai, Kaunas distr., Lithuania
E-mail: n.rasiukeviciute@lsdi.lt

Abstract

Botrytis cinerea Pers.: Fr is an important strawberry disease-causing pathogen with a broad host range. Classical *B. cinerea* identification is complicated due to the lack of morphological polymorphism between species. The use of molecular tools helps to identify pathogens fast and accurately. This study aimed to determine *Botrytis* spp. isolates and evaluate the genetic diversity of grey mould population in Lithuania. During June–August of 2012–2014, 273 isolates were sampled from 12 different areas of Lithuania. All samples were isolated from infected fruits, and single-spore isolates were extracted. *B. cinerea* isolates were identified using *B. cinerea* species-specific primers $Bc108^{+}$ / $Bc563^{-}$. The polymerase chain reaction (PCR) showed two bands characteristic of two specific DNA fragments of *B. cinerea* – upper and lower band of 360 and 480 bp, respectively. These two bands reflect pathogen genotype differentiation and could be used for cryptic species detection. The cryptic species analysis revealed that resistant group I accounted for 16.95% and sensitive group II for 83.05% of the Lithuanian collection of *B. cinerea* isolates. The precise identification of the *B. cinerea* cryptic species is important for the species-specific fungicide resistance and aggressiveness. Four microsatellite markers used in this study revealed genetic diversity of *B. cinerea.* The 158 isolates were identified as *B. cinerea*. The most polymorphic microsatellite marker was BC6 (0.88) and the least polymorphic – BC7 (0.79). The isolates clustered into three genetic groups. The first group consisted of 45 strains, the second group of 15 and the third group of 4 isolates. Our data show genetic diversity within the Lithuanian population of *B. cinerea*. One of the management tools is recognition and identification of the pathogen which leads to optimal and efficient disease management.

Key words: grey mould, identification, pathogen, polymorphism.

Introduction

The strawberry is one of the most popular berry fruits in the world, including Lithuania. Winter hardiness is one of the limiting factor affecting plants overwintering and productivity. Despite, due to ripening time and fungal pathogens, strawberry decay losses can reach up to 40% or more (Uselis et al., 2008; Daugaard, 2014; Rugienius et al., 2016).

Fungal pathogens feature several life cycles and often occur in large parts of the world. The necrotrophic *Botrytis cinerea* Pers.: Fr. is one of the most important strawberry diseases all over the world with a with a broad host range. *Botrytis* spp. comprises 22 species and a large number of host-specific pathogens. *B. cinerea* is a fungal pathogen causing grey mould on more than 586 plant genera. The pathogen has the capability to develop an infection at a temperature range from 2°C to 28°C (optimal 20°C) and leaf wetness periods above 80%, which lasts more than 4 hours. It damages hundreds of plants causing grey mould and infects strawberry leaves, fruits, flowers, petioles, stems and often starts early as blossom blight (Williamson et al., 2007; Fournier, Giraud, 2008; Walker, Fournier, 2013; Carisse, 2016; Dewey, Grant-Downton, 2016; Elad et al., 2016).

B. cinerea is an important strawberry disease-causing pathogen, which reduces yield and causes postharvest losses (Williamson et al., 2007; Elad et al., 2016; Rasiukevičiūtė, 2016). The primary strategy of selecting specific plant protection system is knowledge about the pathogen. The lack of difference among *Botrytis* spp. by classical morphological and cultural methods demands a new solution. Molecular tools help to identify *Botrytis* spp. fast and accurately (Williamson et al., 2007; De Miccolis Angelini et al., 2016; Carisse, 2016; Dewey, Grant-Downton, 2016; Elad, 2016; Elad et al., 2016). The microsatellite with specific genetic markers enables fast evaluation of pathogen polymorphism (Fournier et al., 2003; 2005; Fournier, Giraud, 2008; De Miccolis Angelini et al., 2016; Walker, 2016).

Genetic studies revealed that *B. cinerea* is grouped into two cryptic phylogenetic species. Cryptic species are also related to resistance to the fungicide fenhexamid: group I is resistant, and group II is sensitive. The precise identification of the *B. cinerea* group is important for the species-specific fungicide resistance and aggressiveness (Fournier et al., 2005; Fournier, Giraud, 2008; Fournier et al., 2013; De Miccolis Angelini et al.,

Please use the following format when citing the article:
Rasiukevičiūtė N., Rugienius R., Šikšnianienė J. B. 2018. Genetic diversity of *Botrytis cinerea* from strawberry in Lithuania. Zemdirbyste-Agriculture, 105 (3): 265–270 DOI 10.13080/z-a.2018.105.034

2016; Rasiukevičiūtė, 2016; Walker, 2016). Group I is called *B. pseudocinerea* and group II – *B. cinerea sensu stricto*. They have similar morphological features except that asexual spores in group II are significantly smaller than in group I. They occupy the same environment and often parasitize the same host, but *B. pseudocinerea* is more frequent on dead flower parts in the spring (Fournier et al., 2005; Fournier, Giraud, 2008; Walker et al., 2011; Fournier et al., 2013; Carisse, 2016; Elad, 2016; Elad et al., 2016; Walker, 2016).

The biology of the pathogen comprises a number of interactions between host, pathogen and environment which influence disease development (Carisse, 2016; Elad, 2016; Elad et al., 2016). The populations of *B. cinerea* are highly diverse and deeply structured according to the host plant and the cropping system (Walker, Fournier, 2013). Control of strawberry grey mould is based on integrated plant protection with the use of fungicides. Therefore, it is important to understand the pathogen population structure and distribution for optimal use of fungicides. Valiuškaitė et al. (2010) evaluated *Botrytis* spp. Lithuanian population from different hosts. The research revealed that within *Botrytis* spp. population there was no obvious specificity within the geographical location or plant hosts. The aim of this study was to identify *Botrytis* spp. isolates and evaluate the genetic diversity in grey mould population in Lithuania.

Materials and methods

Isolates. During June–August of 2012–2014, 273 isolates were sampled from 12 different areas of Lithuania (Table 1). All samples were taken from infected strawberry *Fragaria* × *ananassa* Duch. fruits using a sterilized needle; then isolates were cultured 7 days at 22 ± 2°C on potato dextrose agar (PDA) (MERK, Germany). The *Botrytis* spp. growing mycelium was transferred to new PDA and cultured again for 7 days at 22 ± 2°C in the dark. This step was repeated twice to purify the culture, then single-spore isolates were extracted. Single-spore isolate collections were stored on PDA at 4°C.

Table 1. *Botrytis* spp. isolates from strawberry, 2012–2014

Area	Year	Cultivar	Number of isolates
Prienai dist.	2014	Marmolada, Sonata, Felicita	17
Prienai dist., Klebiškis	2014	Unknown	6
Šiauliai dist., Adomiškiai	2014	Malvina	20
Kaunas dist.	2012	Unknown	1
Kaunas	2012	Elkat	4
Kaunas	2013	Elkat	5
Kaunas dist., Babtai	2012	Darselect, Elkat	7
Kaunas dist., Babtai	2013	Darselect, Venta, Elkat	82
Kaunas dist., Babtai	2014	Darselect, Elkat	10
Šiauliai dist., Kantminai	2014	Darselect, Elene, Syria	22
Šiauliai dist., Maniušiai	2014	Sonata, Syria	30
Radviliškis dist., Velžys	2014	Pandora, Sonata	19
Panevėžys dist., Sodeliškiai	2014	Selvik, Rumba, Elkat, Felicita, Filut	19
Kėdainiai dist., Dotnuva	2014	Senga Sengana	7
Kėdainiai dist., Labūnava	2014	Syria, Vikat, Pegasus, Pandora	24
Total			273

DNA isolation. Botrytis spp. single-spore isolates were grown on PDA Petri dishes at 22 ± 2°C in the dark. After 7 days of incubation, germinating mycelia were collected and pulverised in liquid nitrogen. For each of the 273 isolates, DNA was extracted using a Thermo Scientific Genomic DNA Purification Kit (Thermo Fisher Scientific, Lithuania). DNA was dissolved in 100 µl of 1× TE buffer and stored at −20°C. DNA concentration was measured with a NanoDrop 1000 spectrometer (Thermo Fisher Scientific, USA).

Identification. B. cinerea Pers.: Fr isolates were identified using *B. cinerea* species-specific primers $Bc108^{+}$/$Bc563^{-}$ (Rigotti et al., 2006) (Table 2). Samples were incubated in a thermoshaker Grant Bio PHMT (Grant Instruments, UK). Polymerase chain reaction (PCR) amplification was performed in a 20 µl reaction volume containing: 2.5 µl 10× Tag buffer, 2 µl dNTP Mix 2 mM each, 0.1 µl of each primer ($Bc108^{+}$ and $Bc563^{-}$), 1.5 µl 25 mM $MgCl_2$, 1 µl of DNA (adjusted to 200 ng μl^{-1}), 0.1 µl Taq DNA polymerase recombinant, 5 U μL^{-1} (Thermo Fisher Scientific), 12.7 µl DNase/RNase-free water. PCR reactions were performed in a Mastercycler (Eppendorf, Germany). PCR amplification was performed as in Khazaeli et al. (2010). PCR reactions were repeated twice and electrophoresis was resolved on a 1.5 percent agarose in 1× TAE buffer gel and visualised by staining with Ethidium bromide (CarlRoth, Germany). The used marker used is GeneRuler DNA Ladder mix 1 kb (Thermo Fisher Scientific).

Cryptic species. PCR for cryptic species detection was performed with specific primers 262/520L according to the protocol of Fournier et al. (2003) (Table 2). PCR amplification was performed in a 20 µl reaction volume containing 1.5 µl of DNA, 2 µl 10× PCR buffer (Sigma Aldrich, USA), 12.1 µl DNase/RNase-free water, 1 µl $MgCl_2$ (Thermo Fisher Scientific), 0.4 µl 10 mM dNTP (Thermo Fisher Scientific), 1 µl REDTag Genomic DNA Polymerase (concentration 1 unit μL^{-1}), 1 µl of each 262 and 520L primer. PCR reactions were performed in a Mastercycler (Eppendorf). The program applied for amplification was as follows: 1 cycle of 5 min at 94°C, 35 cycles of 30 s at 94°C, 1 min 30 s at 55°C, 1 min at 72°C; 1 cycle of final extension for 5 min at 72°C (Fournier et al., 2003). Digestion was made directly after PCR amplification. The reaction volume was 31 µl containing: 10 µl of PCR reaction mixture, 18 µl DNase/RNase-free water, 2 µl 10× Tango buffer (Thermo Fisher Scientific), 1 µl HhaI (10 U μl^{-1}) enzyme (Thermo Fisher Scientific). Digestion was made in water-thermostat (Biosan, Latvia) for 2 hours at 37°C and directly after digestion reaction was inactivated with 1.24 µl 0.5 M EDTA (20 nM final concentration). The product was amplified at 601 bp (group I) and 517 bp (group II) (Fournier et al., 2003). PCR reactions were repeated

Table 2. Primer sequences used for analysis of *Botrytis cinerea* microsatellites

Primer	Primer sequence	Size bp	Reference
$Bc108^+$ $Bc563^-$	F 5'-ACCCGCACCTAATTCGTCAAC-3' R 5'-GGGTCTTCGATACGGGAGAA-3'	360, 480	Rigotti et al., 2006
Bc2-F Bc2-R	FAM 5'CATACACGTATTTCTTCCAA3' 5'TTTACGAGTGTTTTTGTTAG3'	200	Fournier et al., 2002
Bc6-F Bc6-R	HEX 5'ACTAGATTCGAGATTCAGTT3' 5'AAGGTGGTATGAGCGGTTTA3'	100, 150, 300	
Bc7-F Bc7-R	TAMRA 5'CCAGTTTCGAGGAGGTCCAC3' 5'GCCTTAGCGGATGTGAGGTA3'	150	
Bc10-F Bc10-R	ROX 5'TCCTCTTCCCTCCCATCAAC3' 5'GGATCTGCGTGGTTATGACG3'	160, 200	
262 520L	5'AAGCCCTTCGATGTCTTGGA3' 5'ACGGATTCCGAACTAAGTAA3'	1172	Fournier et al., 2003

twice and electrophoresis resolved on a 1.5 percent agarose in 1× TAE buffer gel and visualised by staining with Ethidium bromide (CarlRoth, Germany). The used marker used is GeneRuler DNA Ladder mix 1 kb Ladder (Thermo Fisher Scientific).

Microsatellite analysis. Microsatellite single sequence repeat (SSR) analysis was performed using four of the nine microsatellite markers developed by Fournier et al. (2002). PCR amplifications were multiplexed. Microsatellite PCR amplification was performed in a 10 µl reaction volume containing 1 µl of DNA, 1 µl PVP, 0.1 µl DDT, 1 µl 10× Tag buffer, 1 µl dNTP Mix 2 mM each, 0.7 µl 25 mM $MgCl_2$, 0.05 µl Taq DNA polymerase (concentration 5U μL^{-1}, recombinant) (Thermo Fisher Scientific), 4.65 µl DNase/RNase-free water, 0.5 µl of primer mix 1 or 2. Primer mix 1: BC2 and BC6; primer mix 2: BC7 and BC10 (Table 2). PCR reactions were performed in a Mastercycler (Eppendorf). The program applied for amplification was as follows: 1 cycle of 5 min at 95°C, 28 cycles of 30 s at 95°C, 90 s at 59°C, 30 s at 72°C; 1 cycle of final extension for 30 min at 60°C (Fournier et al., 2003).

Fragments were separated in an automated single-capillary genetic analyser sequencer. Fragment analysis was performed using genetic analyser 3130 (Applied Biosystems, USA) using 36 cm capillary array and POP-7 polymer. Data were analysed using software *GeneMapper*, v.4.0 (Applied Biosystems).

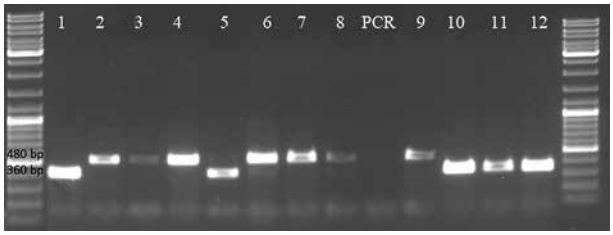
Statistical analysis. The genetic distance matrix was calculated using the Nei and Li (1979) method and cluster analysis was conducted using the UPGMA (unweighted pair-group method with arithmetic mean) method. To test the reliability of the dendrograms, a bootstrap analysis with 1000 replications was performed.

Results and discussion

Pathogen identification. Based on phenotypical, ecological and genetic description, *Botrytis cinerea* is known as a highly variable species (Carisse, 2016; De Miccolis Angelini et al., 2016; Elad et al., 2016). The present study, using genetic markers, confirmed the occurrence of *B. cinerea* in Lithuania. The accurate identification and monitoring of the pathogen help to control it (De Miccolis Angelini et al., 2016; Elad, 2016; Elad et al., 2016; Rasiukevičiūtė, 2016). Species-specific primers have been developed for *B. cinerea* detection (Rigotti et al., 2002; 2006). Several types of research revealed that *B. cinerea* population is extremely diverse (Fournier et al., 2003; 2005; Fournier, Giraud, 2008; Carisse, 2016; De Miccolis Angelini et al., 2016; Elad et al., 2016; Walker, 2016).

Therefore, in this study more than 270 *Botrytis* spp. samples from 12 different areas of Lithuania, collected in 2012–2014, were analysed to identify as *B. cinerea.* Fungi were isolated from infected strawberry fruits. The PCR is one of the methods which allow accurate species identification. Identifying species of fungi, the known DNA sequences can be used. Rigotti et al. (2002; 2006) have designed several specific primers, but in our study $Bc108^+/Bc563^-$ was more accurate because it amplifies two different-size fragments.

The PCR of 273 isolates resulted that only 158 were identified as *B. cinerea* (Fig. 1). The primers $Bc108^+/Bc563^-$ gave two bands of DNA fragment of *B. cinerea*, upper band 360 bp or lower 480 bp. These two bands reflect pathogen genotype differentiation and could be used for cryptic species detection (Rasiukevičiūtė, 2016). The data suggested that 7.59% of isolates belong to group I and 92.41% – to group II. These two groups reflect the pathogen response to the fungicide fenhexamid. Our data display that Rigotti et al. (2006) $Bc108^+/Bc563^-$ primers confirm not only *B. cinerea* but also specify cryptic species. Our data show that molecular markers are more useful in the detection of *B. cinerea* species. The molecular tools using species-specific primers helped us to identify isolates of *B. cinerea* precisely and rapidly.



Amplified fragment 360 and 480 bp; 1–12 – *B. cinerea* fragment, PCR – negative control (reaction mixture without pathogen DNA)

Figure 1. The electrophoretic profile of polymerase chain reaction (PCR) products of *Botrytis cinerea* isolates, obtained with primers $BC108^+/BC563^-$

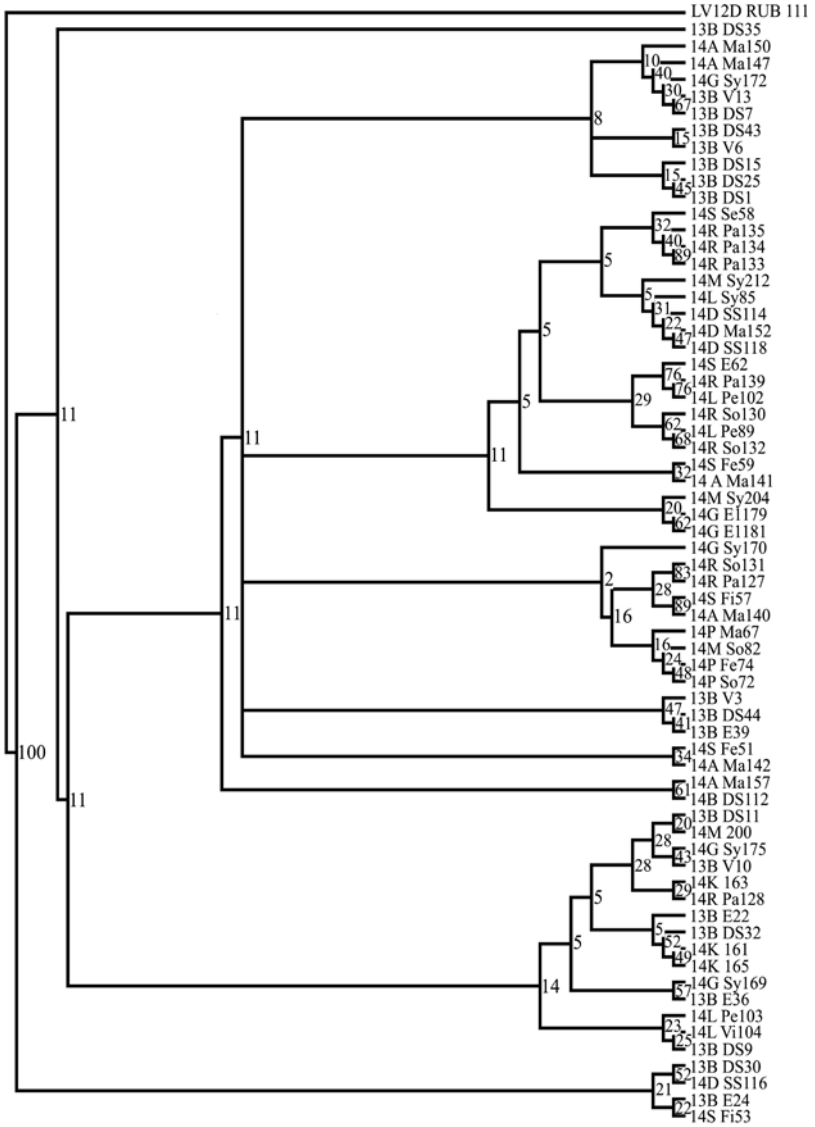
We selected 59 isolates obtained from our study to identify these two distinct *B. cinerea* groups. The size of amplified group I band was 601 bp and for group II – 517 bp. Isolates of group I are resistant to fenhexamid, whereas those of group II are sensitive (De Miccolis Angelini et al., 2016; Walker, 2016). Fournier et al. (2005) determined that group I and II represent *B. cinerea* cryptic species. Walker et al. (2011) have reported that group I is new species of *Botrytis* spp. named *B. pseudocinerea*. Our data revealed that among the 59 isolates predominant were sensitive to fenhexamid

group II – 83.05% isolates and resistant group I – only 16.95% isolates (Table 3). Our data agree with other authors' findings that group II is predominant on infected fruits (Fournier et al., 2005; Wahab, 2015; Saito et al., 2016). Saito et al. (2016) found that 99.3% of isolates belonged to group II and only 0.7% – to group I. Martinez et al. (2008) also suggest that group II (97.7%) is highly dominant over group I (2.3%). Our data show that group II is prevailing in Lithuanian isolates. However, in our study we observed slightly more group I isolates than in the studies mentioned above. It is likely that the ratio of those groups is changing over time, due to intensive use of pesticides. Further studies and monitoring are required.

Table 3. Cryptic species of *Botrytis cinerea*, group I and II

Origin	Number of isolates	Group	
		I	II
Babtai	46	9	37
Kaunas	9	0	9
Kaunas distr.	4	1	3
Total	59	10	49

Microsatellite analysis. A group of 67 *B. cinerea* isolates from different areas of Lithuania were analysed. All 67 *B. cinerea* isolates were completely genotyped using four microsatellites (Table 5). All examined microsatellite loci were polymorphic. The four microsatellite markers exhibited between 9 and 23 alleles, with an average of 13.25 alleles per marker in the analysed *B. cinerea* samples (Table 4). Fournier and Giraud (2008) found that the number of alleles varied from 3 to 6. The most polymorphic microsatellite marker was BC6 (PIC = 0.88) and the least polymorphic was BC7 (PIC = 0.79). Our data show that polymorphism information content (PIC) was higher than in the study of Leyronas et al. (2015) (PIC = 0.71). The observed heterozygosity varied from 0.02 to 0.61 with the average value of 0.24. Expected heterozygosity value ranged from 0.81 to 0.89 with the average of 0.84. Observed heterozygosity values were lower than expected heterozygosity in all investigated loci (Table 5). Compared with other studies, the mean expected heterozygosity was similar (H_0 = 0.894) to the one reported by Fekete et al. (2012) and higher (H_0 = 0.52–0.79) than that reported by Fournier and Giraud (2008), and by Assadollahi et al. (2013)



Note. Bootstrap frequencies were calculated with 1000 replicates.

Figure 2. Dendrogram of strawbery isolates from different agroecological regions, constructed using UPGMA algorithm

(H_0 = 3.53). Fournier et al. (2013) found that observed heterozygosity ranged from 0.41 to 0.58, which agrees with our results. Lower than expected heterozygosity shows high inbreeding occurrence among isolates. On the other hand, the high expected heterozygosity coincides with high genetic diversity of *B. cinerea* (Kumari et al., 2014; Corwin et al., 2016).

Table 5. Characterization of alleles from different agroecological regions of Lithuania

No.	Marker	Number of alleles	Allele size range bp	H_0	H_e	PIC
1	BC2	9	144–174	0.83	0.02	0.81
3	BC6	23	84–268	0.89	0.61	0.88
4	BC7	12	109–133	0.81	0.21	0.79
5	BC10	9	162–191	0.82	0.12	0.80
Mean		13.25		0.84	0.24	0.82

H_0 – expected heterozygosity, H_e – observed heterozygosity, PIC – polymorphism information content

The dendrogram constructed by UPGMA illustrates the relationship among *B. cinerea* isolates, they were genotyped entirely using four microsatellite primer pairs (Fig. 2). The similarity between the isolates was computed as Nei's genetic identity (Nei, Li, 1979). Isolates tended to cluster into several groups.

The clustering of *B. cinerea* isolates from different regions of Lithuania was constructed using 67 different fragments. The *B. cinerea* strains clustered in three main clades, but two isolates were distant. One of the distant isolates was obtained from raspberry cultivar 'Gerakl' (LV12DRUB111) from Latvia. The second distant was from Babtai, isolated from strawberry cultivar 'Darselect' (13BDS35) in 2013. The first group consisted of 45 strains, the second group of 15 and the third group of 4 isolates. This one cluster (13BDS30, 14DSS116, 13BE24 and 14SFi53) is also quite distant from other two clusters. Fournier et al. (2013) have indicated that geographical origin of the isolates does not alternate genetic diversity of *B. cinerea*. Walker and Fournier (2013) found that genetic variation of *B. cinerea* from grapevine was structured by the cropping system combined with the host plant.

The data of our study show genetic diversity within the Lithuanian population of *B. cinerea*. The literature review also shows high genetic diversity of *Botrytis* spp., but the proportion of isolates sensitive and resistant to fenhexamid differs (Corwin et al., 2016; De Miccolis Angelini et al., 2016; Elad et al., 2016). The importance of *B. cinerea* as strawberry pathogen and its impact on yield require precise management. One of the management tools is recognition and identification of the pathogen. In Lithuania, the use of fenhexamid has never been allowed for strawberry disease control, but despite that our data show that *B. cinerea* group sensitive to it is prevailing in Lithuanian isolates. In our opinion, and according to the literature review, the pathogen could spread out with propagation material which could be from countries where this fungicide is allowed (Elad et al., 2016). Transfer of *B. cinerea* can be indirectly confirmed by the results of our research where some genetically distant isolates were detected (Fig. 2). Furthermore, the cryptic species of pathogen might differ in general or complex resistance to several pesticides. The molecular tools are an important implement showing that analyses of resistance of various host plants to different active ingredients of fungicides must be continued.

Conclusions

1. Polymerase chain reaction confirmed that among 273 isolates of *Botrytis* spp. 158 were identified as *Botrytis cinerea* Pers.: Fr. The primers $BC108^+/BC563^-$ are suitable not only for *B. cinerea* identification, but also for the detection of cryptic species.

2. Assessments of genetic diversity using microsatellite analysis revealed distinction within the Lithuanian population of *B. cinerea*. Polymorphic information values varied from 0.79 to 0.88 with an average of 0.82. The *B. cinerea* strains clustered in three main groups with genetically distinct isolates.

Received 21 12 2017
Accepted 26 03 2018

References

1. Assadollahi M., Fekete E., Karaffa L., Flipphi M., Árnyasi M., Esmaeili M., Váczy K. Z., Sándor E. 2013. Comparison of *Botrytis cinerea* populations isolated from two open-field cultivated host plants. Microbiological Research, 168 (6): 379–388. https://doi.org/10.1016/j.micres.2012.12.008
2. Carisse O. 2016. Epidemiology and aerobiology of *Botrytis* spp. Fillinger S., Elad Y. (eds). *Botrytis* – the fungus, the pathogen and its management in agricultural systems. Springer, p. 127–148. https://doi.org/10.1007/978-3-319-23371-0_7
3. Corwin J. A., Subedy A., Eshbaugh R., Kliebenstein D. J. 2016. Expansive phenotypic landscape of *Botrytis cinerea* shows differential contribution of genetic diversity and plasticity. Molecular Plant-Microbe Interactions, 29 (4): 287–298. https://doi.org/10.1094/MPMI-09-15-0196-R
4. Daugaard H. 2000. Effect of cultural methods on the occurrence of grey mould (*Botrytis cinerea* Pers.) in strawberries. Biological Agriculture and Horticulture, 18 (1): 77–83. https://doi.org/10.1080/01448765.2000.9754866
5. De Miccolis Angelini R. M., Pollastro S., Faretra F. 2016. Genetics of *Botrytis cinerea*. Fillinger S., Elad Y. (eds). *Botrytis* – the fungus, the pathogen and its management in agricultural systems. Springer, p. 35–53. https://doi.org/10.1007/978-3-319-23371-0_3
6. Dewey (Molly) F. M., Grant-Downton R. 2016. *Botrytis* – biology, detection and quantification. Fillinger S., Elad Y. (eds). *Botrytis* – the fungus, the pathogen and its management in agricultural systems. Springer, p. 17–34.
7. Elad Y. 2016. Cultural and integrated control of *Botrytis*. Fillinger S., Elad Y. (eds). *Botrytis* – the fungus, the pathogen and its management in agricultural systems. Springer, p. 149–164. https://doi.org/10.1007/978-3-319-23371-0_8
8. Elad Y., Vivier M., Fillinger S. 2016. *Botrytis*, the good, the bad and the ugly. Fillinger S., Elad Y. (eds). *Botrytis* – the fungus, the pathogen and its management in agricultural systems. Springer, p. 1–15. https://doi.org/10.1007/978-3-319-23371-0_1
9. Fekete E., Fekete E., Irinyi L., Karaffa L., Arnyasi M., Asadollagi M., Sandor E. 2012. Genetic diversity of a *Botrytis cinerea* cryptic species complex in Hungary. Microbiological Research, 167 (5): 283–291. https://doi.org/10.1016/j.micres.2011.10.006
10. Fournier E., Giraud T. 2008. Sympatric genetic differentiation of a generalist pathogenic fungus, *Botrytis cinerea*, on two different host plants, grapevine and bramble. Journal of Evolutionary Biology, 21 (1): 122–132. https://doi.org/10.1111/j.1420-9101.2007.01462.x
11. Fournier E., Giraud T., Loiseau A., Vautrin D., Estoup A., Solignac M., Cornuet J. M., Brygoo Y. 2002. Characterization of nine polymorphic microsatellite loci in the fungus *Botrytis cinerea* (Ascomycota). Molecular Ecology Notes, 2: 253–255. https://doi.org/10.1046/j.1471-8286.2002.00207.x
12. Fournier E., Levis C., Fortini D., Leroux P., Giraud T., Brygoo Y. 2003. Characterization of Bc-hch, the *Botrytis cinerea* homolog of the *Neurospora crassa* het-c vegetative incompatibility locus, and its use as a population marker. Mycologia, 95 (2): 251–261. https://doi.org/10.1080/15572536.2004.11833110

13. Fournier E., Giraud T., Albertini A., Brygoo Y. 2005. Partition of the *Botrytis cinerea* complex in France using multiple gene genealogies. Mycologia, 97 (6): 1251–1267. https://doi.org/10.1080/15572536.2006.11832734
14. Fournier E., Gladieux P., Giraud T. 2013. The 'Dr Jekyll and Mr Hyde fungus': noble rot versus gray mold symptoms of *Botrytis cinerea* on grapes. Evolutionary Applications, 6 (6): 960–969. https://doi.org/10.1111/eva.12079
15. Khazaeli P., Zamanizadeh H., Morid B., Bayat H. 2010. Morphological and molecular identification of *Botrytis cinerea* causal agent of gray mold in rose greenhouses in Central Regions of Iran. International Journal of Agricultural Science and Research, 1 (1): 19–24.
16. Kumari S., Tayal P., Sharma E., Kapoor R. 2014. Analyses of genetic and pathogenic variability among *Botrytis cinerea* isolates. Microbiological Research, 169 (11): 862–872. https://doi.org/10.1016/j.micres.2014.02.012
17. Leyronas C., Halkett F., Nicot P. C. 2015. Relationship between the genetic characteristics of *Botrytis* sp. airborne inoculum and meteorological parameters, seasons and the origin of air masses. Aerobiologia, 31 (3): 367–380. https://doi.org/10.1007/s10453-015-9370-x
18. Martinez F., Corio-Costet M. F., Levis C., Coarer M., Fermaud M. 2008. New PCR primers applied to characterize distribution of *Botrytis cinerea* populations in French vineyards. Vitis, 47 (4): 217–226.
19. Nei M., Li W. H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences, 76 (10): 5269–5273. https://doi.org/10.1073/pnas.76.10.5269
20. Rasiukevičiūtė N. 2016. Genetic and phenotypic diversity of *Botrytis* spp. from strawberry and onion, diseases forecasting and control: doctoral dissertation. Lithuanian Research Centre for Agriculture and Forestry, 189 p. (in Lithuanian).
21. Rugienius R., Šnipaitiene L., Stanienė G., Šikšnianienė J. B., Haimi P., Baniulis D., Frercks B., Pranckietis V., Lukoševičiūtė V., Stanys V. 2016. Cold acclimation efficiency of different *Prunus* and *Fragaria* species and cultivars *in vitro*. Zemdirbyste-Agriculture, 103 (2): 207–214. https://doi.org/10.13080/z-a.2016.103.027
22. Rigotti S., Gindro K., Richter H., Viret O. 2002. Characterization of molecular markers for specific and sensitive detection of *Botrytis cinerea* Pers.: Fr. in strawberry (*Fragaria* × *ananassa* Duch.) using PCR. FEMS Microbiology Letters, 209: 169–174. https://doi.org/10.1016/S0378-1097(02)00491-3
23. Rigotti S., Viret O., Gindro K. 2006. Two new primers highly specific for the detection of *Botrytis cinerea* Pers.: Fr. Phytopathologia Mediterranea, 45: 253–260.
24. Saito S., Margosan D., Michailides T. J., Xiao C. L. 2016. *Botrytis californica*, a new cryptic species in the *B. cinerea* species complex causing gray mold in blueberries and table grapes. Mycologia, 108 (2): 330–343. https://doi.org/10.3852/15-165
25. Uselis N., Lanauskas J., Zalatorius V., Duchovskis P., Brazaitytė A., Urbonavičiūtė A. 2008. Evaluation of the methods of soil cultivation growing dessert strawberries in beds. Sodininkystė ir daržininkystė, 27 (2): 295–305.
26. Valiuškaitė A., Survilienė E., Baniulis D. 2010. Genetic diversity and pathogenicity traits of *Botrytis* spp. isolated from horticultural hosts. Zemdirbyste-Agriculture, 97 (4): 85–90.
27. Wahab H. A. 2015. Characterization of Egyptian *Botrytis cinerea* isolates from different host plants. Advances in Microbiology, 5: 177–189. https://doi.org/10.4236/aim.2015.53017
28. Walker A. S. 2016. Diversity within and between species of *Botrytis*. Fillinger S., Elad Y. (eds). *Botrytis* – the fungus, the pathogen and its management in agricultural systems. Springer, p. 91–125. https://doi.org/10.1007/978-3-319-23371-0_6
29. Walker A. S., Fournier E. 2013. Habitat- and host-specific differentiation in the multihost pathogen *Botrytis cinerea* and evidence for fungicide selection in populations. Dehne H. W. et al. (eds). Modern Fungicides and Antifungal Compounds VII. Deutsche Phytomedizinische Gesellschaft, Braunschweig, p. 167–172.
30. Walker A. S., Gautier A., Confais J., Martinho D., Viaud M., Le Pêcheur P., Dupont J., Fournier E. 2011. *Botrytis pseudocinerea*, a new cryptic species causing gray mold in French vineyards in sympatry with *Botrytis cinerea*. Phytopathology, 101: 1433–1445. https://doi.org/10.1094/PHYTO-04-11-0104
31. Williamson B., Tudzynski B., Tudzynski P., Van Kan I. A. L. 2007. *Botrytis cinerea*: the cause of grey mould disease. Molecular Plant Pathology, 8 (5): 561–580. https://doi.org/10.1111/j.1364-3703.2007.00417.x

ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 105, No. 3 (2018), p. 265–270
DOI 10.13080/z-a.2018.105.034

Botrytis cinerea patogeno, išskirto iš braškių, genetinė įvairovė Lietuvoje

N. Rasiukevičiūtė, R. Rugienius, J. B. Šikšnianienė

Lietuvos agrarinių ir miškų mokslų centro Sodininkystės ir daržininkystės institutas

Santrauka

Botrytis cinerea Pers.: Fr yra svarbus patogenas, sukeliantis braškių ligas, turintis daug augalų šeimininkų. Tradicinis *B. cinerea* indentifikavimas ne visada yra tikslus dėl patogeno rūšių morfologinių požymių panašumo, todėl pastaruoju metu, siekiant jį tiksliau apibūdinti, atliekami molekuliniai tyrimai. Tyrimo tikslas – identifikuoti *Botrytis* spp. izoliatus ir įvertinti jų genetinius skirtumus kekerinio puvinio populiacijoje Lietuvoje. 2012–2014 m. birželio–rugpjūčio mėnesiais surinkti 273 izoliatai iš dvylikos Lietuvos vietovių. Visi mėginiai išskirti iš patogeno pažeistų uogų, ir iš jų išskirtos izoliatų monokultūros. *B. cinerea* identifikuoti parinkti rūšiai būdingi pradmenys $Bc108^{+}$ ir $Bc563^{-}$. Atlikus izoliatų polimerazės grandininės reakcijos (PGR) analizę gauti du *B. cinerea* rūšiai būdingi DNR fragmentai, kurie amplifikavo 360 ir 480 bp fragmentus. Šie fragmentai atskleidžia patogeno genotipo skirtumus ir gali būti naudojami siekiant nustatyti filogenetinių grupes. Filogenetinių tyrimų analizė parodė, kad *B. cinerea* lietuviškoje izoliatų kolekcijoje atsparių I grupės buvo 16,95 %, jautrių II grupės – 83,05 %. Tikslus *B. cinerea* filogenetinių grupių nustatymas yra svarbus dėl rūšies atsparumo tam tikriems fungicidams ir jo agresyvumo. Mikrosatelitų analizė atlikta naudojant 4 skirtingas pradmenų poras. Nustatyta, kad 158 izoliatai yra *B. cinerea*. Pats informatyviausias pradmuo buvo BC6 (0,88), mažiausiai informatyvus – BC7 (0,79). Dendrogramoje izoliatai pasiskirstė į tris pagrindines grupes. Pirmoje grupėje esmingai atsiskyrė 45, antroje – 15, trečioje – 4 izoliatai. Tyrimo duomenys rodo, kad *B. cinerea* populiacijai Lietuvoje yra būdinga genetinė įvairovė. Viena iš patogeno kontrolės priemonių yra jo atpažinimas ir tikslus identifikavimas.

Reikšminiai žodžiai: identifikavimas, kekerinis puvinys, patogenas, polimorfizmas.