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GENETIC VARIATION OF *SCLEROTINIA SCLEROTIORUM* ISOLATES FROM DIFFERENT CONDITIONS

GENETICKÁ VARIABILITA IZOLÁTOV *SCLEROTINIA SCLEROTIORUM* POCHÁDZAJÚCICH Z ODLIŠNÝCH PODMIENOK

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Sclerotinia sclerotiorum (Lib.) de Bary is non-specific and polyphagus plant pathogen with an unusually wide host range. Indeed, it is pathogenic to more than four hundreds of plant species including many agricultural crops and also weed species at the different developmental stages [1, 2]. The economic impact of losses due to sclerotinia diseases is extremely important when pathogen finds adequate conditions for development, such as low temperatures, high relative air humidity, and loamy soil retaining water during winter time.

S. sclerotiorum has been studied for more than 150 years and much knowledge have been undertaken concerning its biology, symptomathology, pathogenicity, and morphology. These efforts have provided much information but little is still known about its genetics, identification, and comparison. *S. sclerotiorum* is a non-specific pathogen whose wide host range provides opportunities for frequent movement between host species. Moreover Carpenter et al. [10] detected no evidence for greater similarity between *S. sclerotiorum* isolates infecting the same host species compared to isolates from different hosts.

Differentiation of *S. sclerotiorum* strains based generally on morphological differences in sclerotia, mycelial growth, and ascospores [3, 4, 5]. However, since no reliable method of identification and differentiation of strains have been developed, molecular techniques focusing on genetic basis should provide progress [6]. Currently, molecular biology approaches enable to evaluate similarity and differences between different isolates within species. Molecular markers generated by different modifications of DNA analyses seem to be effective tool to reveal distinctness between strains within plant pathogens.

The aim of this study was to compare isolates of *S. screrotiorum* originated from completely different agroclimatic conditions moreover collected from different plant species, and to analyse similarity or differences between them based on geographical and plant origin, respectively.

Key words: *Sclerotinia sclerotiorum*, isolates, genetic variation, DNA, PCR

MATERIAL AND METHODS

There were used six different isolates of the *S. sclerotiorum* originated from Algeria and Slovakia, and isolated from various host species (tab. 1). Fungal isolates were maintained on malt-extract agar medium (2.5% malt extract, 2% agar) according to Noonan et al. [6].

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Table 1

Origin of *Sclerotinia sclerotiorum* isolates

Code of isolates	Origin	Host plant
Isolate 1	Bab Ezzouar (ALG)	*Phaseolus vulgaris*
Isolate 2	El-Harrach (ALG)	*Lactuca sativa*
Isolate 6	sea cost (ALG)	*Lycopersicum esculentum*
Isolate 3	Nýrovce (SK)	*Helianthus annuus*
Isolate 4	Veľký Kamenec (SK)	*Conium maculatum*
Isolate 5	Veľký Cetín (SK)	*Iva xanthifolia*

DNA was extracted by method of Della-porta et al. [7] from mycelium growing in liquid potato dextrose broth (2.7% PDB, 1% yeast extract).

PCR reactions consisted of 1.5 mmol.l^{-1} $MgCl_2$, 0.25 mmol.l^{-1} each of dNTPs, 1 mmol.l^{-1} primer, 0.8 U Taq DNA-polymerase, and 25 ng of fungal DNA. Amplifications were performed using 17 different minisatellite, microsatellite, and ISSR primers (tab. 2) using 45 cycles: 1 min at 94°C, 1 min at annealing temperature (each G/C in primer sequence = 4°C, each A/T = 2°C), and 5 min at 72°C. PCR products were separated in 1.5% agarose gels and stained by ethidium bromide.

Statistical analyses have been performed by SPSS 8.0.1 statistical software package (SPSS, Inc.). The Jaccard's similarity coefficients have been calculated and average linkage method between groups has been used for dendrogram construction.

RESULTS AND DISCUSSION

Seventeen used primers generated altogether 166 distinctive markers. Jaccard's coefficients of genetic similarity based on DNA variation generated by all used markers revealed relative high genetic differences between analyzed isolates and ranged from 0.125 to 0.548 (tab. 3). None of isolates were identical by DNA typing but high genetic dissimilarity within isolates did not confirm distinct separation into two groups according to their origin from different geographical and agro-climatic conditions, as could be expected. Reversely some isolates differing by geographical origin were genetically more similar than isolates from the same territory. The most similar were isolates from *Phaseolus vulgaris* (ALG) and *Helianthus annuus* (SK) (Isolates 1 and 3) even though there is no taxonomical relatedness of both host species, and isolates from *Lycopersicum esculentum* (ALG) and from *Iva xanthifolia* (SK) (Isolates 5 and 6). On the contrary

Table 2

Sequences of used DNA primers

Primer code	Primer sequence	Type of sequence	Reference
LBHB01	5'-$(ACTG)_4$-3'	microsatellite	–
LBHB02	5'-$(GACA)_4$-3'	-//-	–
LBHB03	5'-$(GATA)_4$-3'	-//-	–
LBMB-A	5'-$(GACA)_4$TA-3'	ISSR	–
LBMB-B	5'-$(GACA)_4$TT-3'	-//-	–
LBMB-C	5'-$(GACA)_4$GT-3'	-//-	–
HVR^-	5'-CCCTCCTCCTCCTTC-3'	minisatellite	Winberg et al. (1993) [14]
HVR^+	5'-AGGAGGAGGGGAAGG-3'	-//-	Winberg et al. (1993) [14]
YNZ22	5'-CTCTGGGTGTGGTGC-3'	-//-	Nakamura et al. (1987) [15]
FVIIex8	5'-ATGCACACACACAGG-3'	-//-	Murray et al. (1988) [16]
HBV5	5'-GGTGTAGAGAGGGGT-3'	-//-	Nakamura et al. (1987) [15]
HBV3	5'-GGTGAAGCACAGGTG-3'	-//-	Nakamura et al. (1987) [15]
FVIIexB-C	5'-TACGTGTGTGTGTCC-3'	-//-	Murray et al. (1988) [16]
14C2	5'-GGCAGGATTGAAGC-3'	-//-	Vergnaud (1989) [17]
33.6	5'-AGGGCTGGAGGAGGGC-3'	-//-	Jeffreys et al. (1985) [18]
33.15	5'-AGAGGTGGGCAGGTGG-3'	-//-	Jeffreys et al. (1985) [18]
M13 phage	5'-GAGGGTGGXGGXTCT-3'	-//-	Vassart et al. (1987) [19]

T a b l e 3

Jaccard's genetic similarity indexes

	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6
Isolate1		0.125	0.548	0.494	0.151	0.463
Isolate2	0.125		0.125	0.146	0.316	0.241
Isolate3	0.548	0.125		0.531	0.130	0.364
Isolate4	0.494	0.146	0.531		0.160	0.357
Isolate5	0.151	0.316	0.130	0.160		0.548
Isolate6	0.463	0.241	0.364	0.357	0.548	

the most different were Algerian isolates from *Phaseolus vulgaris* and *Lactuca sativa* (Isolates 1 and 2) and isolates from *Lactuca sativa* (ALG) and from *Helianthus annuus* (SK) (Isolates 2 and 3) (fig. 1). The reason of common branching of Isolate 2 (ALG) collected from *Lactuca sativa* and Isolate 5 (SK) collected from *Iva xanthifolia* could be the botanical similarity of the host plants (both belonging to the *Composeae*, *Asteraceae*).

Different studies documented high genetic heterogeneity existing within the *S. sclerotiorum* [3, 8, 9]. C a r p e n t e r et al. [10] studied the genetic variation in New Zealand populations of *S. sclerotiorum* and revealed high variation both within and between populations. M e i n h a r d t et al. [11] used telomere and microsatellite primers and revealed high diversity also among Brazilian isolates of *S. sclerotiorum*. An approach comparable to our has been used by N o o n a n et al. [6] which investigated genetic differences between isolates of *S. sclerotiorum* originated from New Zealand and USA, respectively, by RAPD and rDNA-derived primers. The U.S. isolates originated from the same host plant species, but the New Zealand's ones from 3 different host species. Based on the DNA patterns they allocated analyzed strains into two groups according to country of origin. According to sampling location of *S. sclerotiorum* grouped isolates also S e x t o n and H o w l e t t [12]. No apparent preference of *S. sclerotiorum* isolates to plant host species did not find W i n t o n et al. [13], what also could indicate influence of origin to genetic similarity of isolates.

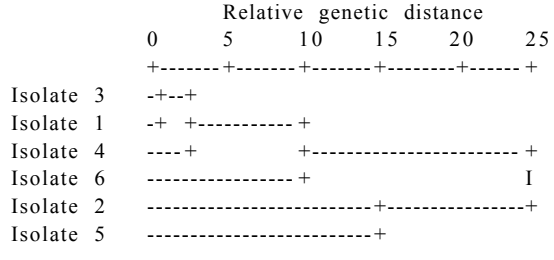

Fig. 1. Relationships within isolates based on DNA analysis

Our study also indicates high inter-origin variation within *S. sclerotiorum* isolates. Although isolates were isolated from six different host plants (lettuce, bean, tomato, poison hemlock, giant sumpweed, and sunflower) the detected genetic variation should indicate high genetic heterogeneity existing within *S. sclerotiorum* isolates. This should be the reason of wide pathogenicity of *S. sclerotiorum* to plant species and high economic impact of this pathogen to plant production.

CONCLUSIONS

Strains of *Sclerotinia sclerotiorum* (Lib.) de Bary isolated from various host plants originated from different agro-climatic conditions has been compared by PCR-based mini- and microsatellite analyses. Variation in DNA banding patterns revealed high variation among differentiated *S. sclerotiorum* isolates and this did not confirm expected separation between geographically distant isolates. Genetic similarity of isolates was higher within host plants originated from different agro-climatic conditions. Obtained data should be an indicative of high genetic heterogeneity existing within *S. sclerotiorum* isolates.

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SÚHRN

Pre sledovanie vnútrodruhovej genetickej variability vybraných izolátov huby *Sclerotinia sclerotiorum* sme použili 17 mikro- a minisatelitných markerov. Izoláty huby boli získané z dvoch geograficky rozdielnych podmienok – Slovenska (z hostiteľských rastlín *Helianthus annuus*, *Conium maculatum* a *Iva Xanthifolia*) a Alžírska (z *Phaseolus vulgaris*, *Lactuca sativa* a *Lycopersicum esculentum*). Molekulárne analýzy ukázali heterogénnosť medzi izolátmi, ale nepotvrdili zreteľné rozdiely medzi izolátmi z geograficky vzdialených podmienok.

Kľúčové slová: *Sclerotinia sclerotiorum*, izoláty, genetická variabilita, DNA, PCR