

**ZASTOSOWANIE METODY LAMP DO OCENY
WYSTĘPOWANIA PIERWOTNIAKA *PLASMODIOPHORA*
BRASSICAE W GLEBACH ROLNICZYCH**

**APPLICATION OF THE LAMP METHOD TO ASSESS THE
PRESENCE OF PROTOZOAN PATHOGEN *PLASMODIOPHORA*
BRASSICAE IN AGRICULTURAL SOILS**

Wśród najnowszych metod powielania kwasów nukleinowych pojawiła się technika LAMP (Loop Mediated Isothermal Amplification), czyli amplifikacja w warunkach izotermicznych z wykorzystaniem starterów zapętlających. Podobnie jak w tradycyjnym PCR synteza nowych nici DNA odbywa się na matrycy, jednakże w trakcie całego jej przebiegu reakcja prowadzona jest w tej samej temperaturze, a przyłączenie starterów i wydłużanie łańcucha DNA nie jest poprzedzone etapem denaturacji. W wyniku reakcji dochodzi do lawinowego powstawania nowych łańcuchów DNA, przy czym powstałe produkty posiadają strukturę składającą się z na przemian odwróconych powtórzeń sekwencji matrycowej. Produkt LAMP różni się zatem zasadniczo od produktu tradycyjnego PCR, bowiem stanowi mieszaninę łańcuchów DNA o podobnej strukturze lecz zróżnicowanej wielkości. Technika LAMP stanowi użyteczne narzędzie do wykrywania i identyfikacji mikroorganizmów chorobotwórczych, w tym patogenów roślin. W niniejszej pracy przy pomocy LAMP badano występowanie pierwotniaka *Plasmodiophora brassicae* w glebach rolniczych w Polsce. Badania prowadzono zarówno dla prób przygotowanych w laboratorium, jak też na próbach uzyskanych z terenu, ze stężeniami patogena uprzednio oznaczonymi metodą Real-Time PCR. Wykonano badania dla 50 prób gleby z terenu Wielkopolski, Dolnego i Górnego Śląska, Małopolski oraz Opolszczyzny. Metoda Real-Time PCR była bardziej czuła, lecz ze względu na jej kosztochłonność i potrzebę stosowania specjalistycznej aparatury technika LAMP ma szansę ją zastąpić w prowadzonych na szeroką skalę badaniach przesiewowych, dla oznaczenia pól, na których uprawa roślin kapustowatych obciążona jest szczególnym ryzykiem.

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eventually enhancing the depletion of polychlorinated biphenyls (PCBs) in historically contaminated sediments ($6.260 \pm 9.3 \text{ } 10^{-3} \mu\text{g PCB/ g dry weight}$). Biostimulation consisted of the amendment to the sediments of the resuscitation promoting factor of the *Kocuria rhizophila* for increasing the possibility to cultivate those bacterial candidates that in natural environment, survive under a wide variety of stress conditions by entering a 'viable but non-culturable' (VBNC) state, in which cells are intact and alive but fail to grow either in their natural environment and on bacteriological media; however, consisting in bacterial candidates possibly exploitable for bioaugmentation of contaminated matrices with a bacterial population with metabolic traits of interest. The effects of the amendment of the extracellular organic matter (EOM) from *Kocuria rhizophila* to the contaminated sediments have been analyzed in terms of (1) the relative abundance of selected bacterial groups with reference to untreated sediments by quantitative real-time PCR, and of (2) the characterisation of pure cultures unique to the enrichment culture deriving from sediments with EOM amendment. The amendment of the extracellular organic matter (EOM) from *Kocuria rhizophila* determined mainly the enrichment of the Actinobacteria. Some isolates belonging to the genus of interest has been isolated, cultivated on bacteriological media and analysed for their capacity to deplete biphenyl in vitro.

PS4-27

INCIDENCE OF PLASMODIOPHORA BRASSICAE AND THE COMPOSITION OF ITS RACES IN SOILS OF POLAND

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Oilseed rape is susceptible to a number of diseases that cause significant economic losses to farmers. Clubroot caused by protozoa species *Plasmodiophora brassicae* Woronin is now one of the biggest threats to healthiness of oilseed rape plants. In Poland the presence of clubroot was reported on 250 thousand hectares of agricultural soils. The pathogen was found in all main growing areas of oilseed rape. The aim of this work was to determine the incidence and recognize races of *P. brassicae* in the soils of Poland. The pathogen was gathered from root tumors of oilseed rape plants in different regions of Poland. The collection of samples included new variants breaking the currently used resistance gene(s). The samples were collected from 67 fields of winter oilseed rape located in 13 provinces of Poland, mainly Pomerania, Varmia & Mazuria, Lower Silesia and Opole region. The tumors were frozen or air-dried and propagated on the susceptible genotype of *B. rapa* var. *pekinensis* variety 'Granaat'. To examine current population of clubroot in Poland we have used reference forms proposed by Somé et al. (2003) as well as cv. Mendel - a cultivars with resistance Crr genes. The assessment was done using 0 to 4 scale, where 0 was a healthy plant with fully developed roots and 4 was a small plant with roots changed to a club. We have determined 7 races of *P. brassicae* in Poland. The most common races were P1 (52%) and P3 (36%). Molecular detection of *P. brassicae* using Real-time PCR showed very high incidence of this microorganism in numerous soils. The incidence of clubroot depended on soil pH, intensity of oilseed rape cultivation as well as soil moisture.

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**MICROBIAL POPULATIONS OF LEPTOSPHERIA SPP. ON F1
BRASSICA HYBRIDS**

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Stem canker of brassicas (blackleg), caused by the fungal complex *Leptosphaeria maculans*-*L. biglobosa* is one of the most destructive diseases of oilseed rape (*Brassica napus*) worldwide. In numerous countries, including Poland both pathogen populations co-exist and they can jointly lead to severe disease symptoms as well as yield losses. The incorporation of *L. maculans* resistance into *Brassica* lines with desirable agronomic and quality traits is a major objectives in breeding programs. At present (1)4 R-genes effective against *L. maculans* have been reported (*Rlm1-Rlm10* and *LepR1-LepR4*) from various *Brassica* species. The aim of this study was to look for the genetic resistance to stem canker in *Brassica* F(1) hybrids in field conditions. The experiment was done in Dłoń (N51o41'22.0", E 17o04'23.0"), Wielkopolska (Great Poland) region. F1 generations of interspecific hybrids between three *B. napus* cultivars and *B. carinata*, *B. fruticulosa*, *B. rapa* ssp. *chinensis*, *B. rapa* ssp. *pekinensis* as well as *B. napus* cultivar with *Rlm7* gene were evaluated. The determination of *Leptosphaeria* species was studied using Loop-mediated DNA Amplification (LAMP) method. For this purpose leaf samples were collected from plants of hybrid lines. Disease incidence was assessed in two seasons (autumn 2014 and spring 2015), on 50 plants per replicate, according to the scale 0-5. The genotypes differed with their reaction to the pathogen. In both seasons, the cultivar with *Rlm7* resistance gene showed significantly less phoma leaf spotting symptoms, as compared to genotypes with no *Rlm7*. The pathogen population of fungi causing blackleg of oilseed rape in Poland was composed of *L. maculans* and *L. biglobosa*. The isolates on leaves of genotypes without *Rlm7* resistance gene were mainly identified as *L. maculans* (83%), whereas the isolates obtained from cultivars harbouring *Rlm7* resistance gene were scarce (8%) and belonged mostly to *L. biglobosa*.

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