Abstract

Poland is one of the key strawberry producers in the world - in 2020, the area under cultivation of this fruit occupied over 30,000 hectares in the country and was smaller only than the area in China. Poland produces almost 200,000 tonnes of this fruit annually, second only to Spain and just before Germany. It is also worth noting that Poland is the leader in organic strawberry production in Europe and in the world.

Organic farming is becoming more and more important in recent years - the European Union presented in 2020 the Farm to Fork Strategy, which is the heart of the European Green Deal. The strategy calls for a significant reduction in the use of chemicals including fungicides and pesticides, while increasing the share of organic farming. However, it should be remembered that the organic way of food production requires the exclusion of many mineral fertilizers and plant protection agents, which makes it much more difficult to maintain a healthy plantation and high yields. In addition, strawberry fruits have a delicate cell wall, which can be easily penetrated by pathogens, which contributes to a decrease in yield and quality of fruits. Therefore, it is essential to monitor the crop quickly and effectively for pathogenic microorganisms in order to use targeted protection measures before the spread of diseases in the plantation. In addition, characterizing the differences of microbiome between healthy and diseased crops will lay a foundation for the development of effective solutions, including biopreparations and production strategies that will increase the health and productivity of soft fruit crops in a sustainable way.

Classical methods of identifying the pathogen that attacks fruit plantations centre mainly around the recognition of disease symptoms on plants, or the observation of morphological characteristics of pure strains of the pathogen isolated from symptomatic plants on microbial media and under a microscope. Importantly, identification of the pathogen carried out in this manner is time-consuming and often inaccurate, and may result in the implementation of inappropriate chemical plant protection products, which unnecessarily burdens the environment and is economically disadvantageous.

In the present doctoral thesis, the most important information concerning characterization, molecular detection methods that were developed so far against key pathogens of strawberry - fungi Botrytis cinerea, Colletotrichum acutatum, Verticillium spp. and funguslike oomycete Phytophthora spp. were collected. Moreover, the thesis includes the international scientific literature review concerning threats to agriculture and food production caused by these phytopathogens.

In the further part of the study, the usefulness of molecular methods of detection of the mentioned key pathogens of strawberry was optimized and demonstrated in order to identify the pathogen in organic plantations. For this purpose, methods based on real-time Polymerase Chain Reaction (real-time PCR) and Loop-Mediated Isothermal Amplification (LAMP) techniques were developed, for which sequences of appropriate molecular markers were designed, reaction reagent concentrations and temperature profile were optimized, ensuring optimal reaction and efficient detection. The methods were tested on genetic material from both, pure pathogen strains isolated from symptomatic strawberry plants, and total DNA isolated from environmental samples (soil, plant parts and fruits). For the developed real-time PCR reaction, the detection limit was 39 fg/µL for the pathogens belonging to the genera of Botrytis and Verticillium, while for Colletotrichum spp. - 156 fg/µL. On the other hand, the detection - 8 - limit of the developed LAMP reaction was 3 pg/µL for Phytophthora spp. and 300 fg/µL for Phytophthora cactorum, indicating the high sensitivity of the optimized methods.

In the next stage of the present study, Illumina Sequencing-by-Synthesis (SBS) nextgeneration sequencing and bioinformatical analyses performed in R, Python and C++ programming languages were also used to characterize the mycobiome of soil, rhizosphere, roots, and shoots of

strawberry and to determine differences in the fungal microbial structure of healthy and infested plantations.

keywords: organic farming, phytopathogen detection methods, real-time Polymerase Chain Reaction (real-time PCR), Loop-Mediated Isothermal Amplification (LAMP), Illumina Sequencing-by-syn.hesis (Illumina SBS), mycobiome