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MOLECULAR DIAGNOSTICS OF SOIL-BORNE PATHOGENIC FUNGI IN SEVERAL AGRICULTURAL CROPS

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Abstract. Modern requirements for product quality provide for expanding the competence in sectors of producing and managing agricultural products by investigating the cause-effect relationships in the transmission of diseases between species through host-pathogen interactions. Monitoring and accurate identification of pathogens in agriculture products are essential for successful implementation of an integrated pest management program. Among the pathogens that have a serious impact on agricultural production, micromycetes outnumber all other species. Diagnostics of fungal pathogens using traditional methods can be difficult due to the similarity of their morphological traits, the lack of the ability to study non-culturable genotypes. In this regard, molecular detection methods based on polymerase chain reaction (PCR) were used in the study, which allow specific detection of any microorganisms. Analysis of the species composition of pathogenic fungi of *Fusarium, Penicillium* and *Aspergillus* genera in the genotypes of some vegetable crops of local selection was conducted based PCR-assay. The efficiency of molecular technique for the species-specific detection of pathogenic fungi that can affect agricultural crops was demonstrated. Molecular diagnostic assessment of the phytosanitary status of seeds of different storage periods was done. Development of some fungal infection during plant ontogenesis using *nested*-PCR assay was monitored.

Key-words: *PCR, Fusarium, Aspergillus, Penicillium, bell pepper, eggplant, beans.*

Rezumat. Cerințele contemporane pentru asigurarea unei calități înalte a produselor agricole impun o extindere a competențelor în domeniul producției și gestionării acestora, prin investigarea relațiilor cauză-efect ce determină transmiterea bolilor între specii, în contextul interacțiunii gazdă-patogen. Monitorizarea precisă și identificarea corectă a agenților patogeni din produsele agricole sunt esențiale pentru implementarea cu succes a unui program de management integrat al dăunătorilor. Printre agenții patogeni cu un impact major asupra producției agricole, micromicetele se disting prin prevalența lor comparativ cu alte specii. Diagnosticul agenților patogeni fungici prin metode tradiționale poate fi dificil datorită asemănării morfologice a acestora și a imposibilității studierii genotipurilor necultivabile pe medii nutritive. În acest context, studiul a utilizat metode de detecție moleculară bazate pe reacția de polimerizare în lanț (PCR), tehnică ce permite determinarea specifică a diverselor microorganisme. Pe baza testului PCR, a fost realizată o analiză detaliată a compoziției speciilor de patogeni din genurile *Fusarium*, *Penicillium* și *Aspergillus*

în genotipurile unor culturi agricole de selecție locală. Rezultatele obținute au demonstrat eficiența tehnicii PCR pentru diagnosticul specific a speciilor fungice patogene care pot afecta culturile agricole. De asemenea, s-a realizat o evaluare moleculară a stării fitosanitare a semințelor, pe parcursul diferitelor perioade de depozitare. A fost monitorizată răspândirea infecției fungice în timpul ontogenezei plantelor, utilizând metoda *nested*-PCR.

Cuvinte-cheie: *PCR, Fusarium, Aspergillus, Penicillium, ardei, vinete, fasole.*

1. Introduction

Providing the growing population with foodstuffs and preserving the environment is a huge problem that humanity encounters on a global scale. Human activity and stressed ecosystems have created new conditions for the emergence and spread of pathogen-induced diseases in the food industry and agricultural sectors, posing a threat to food security. Sustainable production of goods and managing ecosystems are of paramount importance and require a "One Health" approach. "One Health" concept implies that human, animal, plant and environmental health are inextricably connected, which requires interconnection of the respective sectors and not their separation [1].

An integral part of this concept is controlling the pathogenic microorganisms in the environment, monitoring diseases at local, regional, national and global levels. Modern requirements for product quality provide for expanding the competence in sectors of producing and managing agricultural products by investigating the cause-effect relationships in the transmission of diseases between species through host-pathogen interactions.

Plant pathogenic diseases pose a serious threat for crop yield and cause significant economic losses. Most of the strategies for their combating focus on soil. Intensive tillage has a negative impact on soil functioning and, as a consequence, on plant productivity. Long-term use of pesticides leads to loss of their effectiveness and promotes pathogen adaptation and the emergence of new, more aggressive genotypes resistant to antimicrobial agents [2]. To improve soil quality and preserve biodiversity, finding safe approaches for pathogen control is a priority. Integrated pest control requires specific solutions in each particular case. Monitoring and accurate identification of pathogens are essential for successful implementation of an integrated pest management program.

Among the pathogens that have a serious impact on agricultural production, micromycetes outnumber all other species. They are an important component of soil microbiota. However, from a practical point of view, they pose a major problem for agrosystems, causing up to 80% yield losses [3, 4]. In particular, soil fungi of *Fusarium, Penicillium* and *Aspergillus* genera are among the most harmful pathogens that reduce crop yield worldwide [5]. Species of *Fusarium* genus cause various types of wilts, rots, tissue necrosis and vascular diseases during plant vegetation [6]. In addition, most *Fusarium, Penicillium* and *Aspergillus* species produce mycotoxins that are hazardous to human and animal health, the content of which in plant products is strictly regulated [7].

Soil fungal pathogens can survive latently in soil for a long time in the absence of a host plant, forming stable structures. As a result, the diseases they cause are particularly difficult to forecast and successfully control. Screening of pathogenic microbiomes for identifying causal agents of diseases is the first step towards revealing direct and indirect links between the plant and the pathogen. Diagnostics of fungal pathogens using traditional microbiological methods can be difficult due to the similarity of their morphological features, the lack of the ability to study slow growing or non-culturable genotypes. In this regard,

molecular detection methods based on PCR are used in the practice of phytopathology, which allow specific detection of any microorganisms. They accelerate the identification of pathogens, including the cases of complex infection, allow the differentiation of pathogens with identical symptoms, and provide the ability to detect the pathogen at the pre-symptomatic stage. These minimize the risks of dissemination of pathogens.

A PCR-based technology was optimized for identifying various soil-born plant pathogens [8]. This approach was used to analyze species composition of fungi of *Fusarium, Penicillium* and *Aspergillus* genera in the genotypes of some vegetable crops of local selection. Monitoring of causal agents of fungal infections was performed in seeds of different storage periods and in plants at different phases of ontogenesis.

2. Material and methods

2.1. Plant material

The seeds of common bean, bell pepper and eggplant of different storage periods of the collection of Laboratory of Plant Genetic Resources (IGPPP) were analyzed. In the study were used following genotypes, Tabel 1:

Table 1

Description of seeds genotypes

Taxon	Genotype name	Year of preservation	Originator
Common bean (*Phaseolus vulgaris*)	MDS202, MDS204, MDS209	2015, 2020	Local forms from subsistence farms
Bell pepper (*Capsicum annuum*)	Fildes, Caolin, Excelent	2015, 2020	Varieties of Moldavian selection
Eggplant (*Solanum melongena*)	Rada, Magda	2011, 2018	Varieties of Moldavian selection

Eggplant genotypes of local selection cultivated on the experimental plots of the IGPPP served as objects of the field study in current research. Four eggplant genotypes of the collection of Laboratory of Plant Genetic Resources were studied: 'Laura', 'Forma 92', 'Magda' and 'Sucleischii' varieties. Different organs of plants during ontogenesis were tested. The asymptomatic leaves and fruits were selected randomly from 10-12 plants and bulk samples were prepared.

Prior to extraction of nucleic acids, seeds, leaves or fruits were thoroughly washed to remove impurities and surface microflora.

2.2. Desoxyribonucleic acid (DNA) extraction

The PCR reaction requires a pure DNA template in optimal quantities to ensure correct amplification of the target sequence. Nucleic acids were extracted from 0.2 – 0.5 g of seeds or 1 g of plant material and were purified according to the combination of several protocols from ISO 21571:2005 [9,10].

2.3. Primers

The specificity of PCR depends on the correct design of highly specific primers. Primers must be carefully designed to amplify only the genetic sequences of interest and to eliminate the possibility of false positive or negative results.

PCR primer design was done based on species-specific sequences of each pathogen from NCBI nucleotide collection presented in GenBank database using Primer-BLAST designing tool [11,12]:

- Translation elongation factor 1-alpha (*tef1*) gene sequences of *Fusarium verticillioides, Fusarium oxysporum, Fusarium solani, Fusarium avenaceum*.
- Beta-tubulin (*tub2*) gene sequences of *Fusarium equiseti, Fusarium sporotrichioides, Penicillium chrysogenum, Penicillium expansum, Penicillium citrinum*.
- Oxygenase (*fum6*) gene sequences of *Fusarium proliferatum*.
- RNA polymerase II subunit (*RPB2*) gene of *Fusarium nivale*.
- O-methyltransferase A (*aflP*) gene, partial cds; and *aflP-aflQ* intergenic region, genomic sequence of *Aspergillus flavus, Aspergillus parasiticus*.

A theoretical analysis of primer selection and conditions for their use in PCR was performed.

2.4. Polymerase chain reaction (PCR)

PCR is an experimental method of molecular biology suitable for detecting pathogens in DNA extracted from infected plant material excluding the step of their isolation and cultivation.

This highly specific technique allows rapid amplification of a specific DNA fragment of interest in biological material, permitting its detection and visualization. Methods of the molecular diagnostics present the possibility of identifying pathogens that cause diseases with the similar symptoms in mixed infections, as well as before the appearance of visible symptoms. PCR-assay is much faster than conventional culture-based methods, so the results can be obtained within a few hours.

PCR was performed in a 25 µL mix containing 66 mM Tris-HCl (pH 8.4), 16 mM $(NH4)_2SO_4$, 2.5 mM $MgCl_2$, 0.1% Tween 20.7% glycerol, 100 µg/ml-1 BSA, 0.2 mM of each dNTPs, 0.2 U Taq DNA polymerase (Thermo Fisher Scientific), 5 pM of each primer and 10 ng of DNA.

2.5. One-step PCR

The amplification conditions were as follows: in the first cycle, denaturation was carried out at 95^0C for 3 min; annealing – at 60 °C, 40 s, elongation – at 72 °C, 40 s, followed by 34 cycles: 95 °C – 40 s, 60 °C – 40 s, 72 °C – 40 s, final elongation - 7 min. Amplification was carried out using MiniAmp™ Thermal Cycler, 96 wells, Thermo Fisher Scientific.

2.6. Nested-PCR

Nested-PCR is a variant of conventional PCR and consists of two rounds, in which two pairs of primers are used in consecutive reactions. The second pair of primers amplify the DNA region within the product of the first reaction. It is applied to reduce nonspecific amplification and dramatically increases the sensitivity, productivity and specificity of the analysis.

The first round of *nested*-PCR included 1 cycle at 95 °C for 3 min (denaturation), 60 °C – 40 s (annealing), 72 °C – 40 s (elongation) followed by 29 cycles: 95 °C – 40 s, 60 °C – 40 s, 2 °C – 40 s, final elongation - 7 min. The conditions of the second round for *nested*-PCR were: 95 °C – 40 s, 60 °C – 40 s, 72 °C – 40 s, final elongation - 7 min (30 cycles). Amplification was carried out using MiniAmp™ Thermal Cycler, 96 wells, Thermo Fisher Scientific.

2.7. Results processing

The quality analysis of the isolated DNA and separation of the amplification products was performed in 1.5-2% agarose gel in the presence of 100 bp DNA ladder (Thermo Fisher Scientific molecular marker) with the addition of ethidium bromide, 1xTBE (pH 8.0) migration buffer, for approximately 1 h at 6V/cm. Visualization was performed in UV rays at a wavelength of 312 nm and photographed. The target fragments were compared with commercial molecular markers.

3. Results and discussion

3.1. Assessment of DNA quality

The integrity and quantity of total DNA extracted from plant material was carried out by electrophoresis in 1.5% agarose gel in the presence of coliphage λ DNA of known concentration (0.1 μg/10 μL). The results of the assessment of DNA quality are selectively presented in Figure 1.

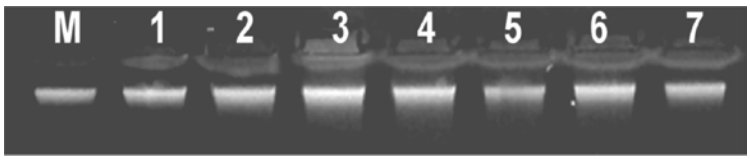

Figure 1. Electropherogram of total DNA extracted from seeds (1-3) and leaves (4-7) of eggplant. **M** - λ DNA - 0.1 μg/10 μL; **1-7** - 10 μL plant DNA per line.

Excessive amounts of DNA in a PCR reaction can suppress amplification. This can also lead to false priming and decreased PCR efficiency. As described in Material and methods (2.4. Polymerase chain reaction), 10 ng of DNA per sample was required for the PCR reaction mix. Based on the electrophoresis results, visually the amount of applied plant DNA (10 μL) is comparable to the amount of coliphage λ DNA (0.1 μg/10 μL). Thus, no more than 1 μL of DNA from each sample was used in subsequent molecular analysis.

The amplification capacity of DNA was validated by one-step PCR using primers designed based on the sequences of plant housekeeping genes, in particularly - 18S ribosomal RNA genes (18SrDNA) [13]. An example of PCR amplification of a target fragment of total DNA isolated from plant material using primers to the 18SrDNA is shown in Figure 2.

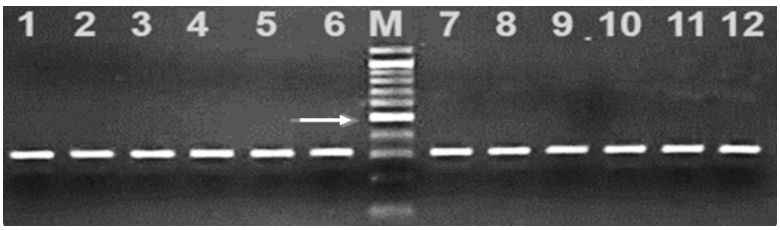

Figure 2. Electropherogram of the PCR products obtained on total DNA extracted from eggplant leaves (**1-12**) using primer to 18SrDNA. **M** - marker (100 bp DNA ladder). The arrow points at a 500 bp marker fragment.

As a result of one-step PCR using the specified primers, a fragment of 315 bp should be synthesized. The presence of amplicons of the expected length indicated that all tested DNA samples were free of PCR inhibitors and suitable for subsequent molecular analysis.

3.2. Validation of primers

Correct detection of fungal pathogens in plant material by molecular methods depends on highly specific primers for each taxon and suitable PCR protocol. Successful amplification is determined by obtaining amplicons corresponding to the target sequence of

certain pathogens and the absence of synthesis of non-specific products. The use of *nested*-PCR significantly improves the efficiency of pathogen detection.

This variant of PCR reduces the risk of non-specific amplification, which can lead to false negative or false positive results. But the sensitivity and specificity of PCR analysis should always be validated in each individual case. Therefore, initially the primers were tested using DNA extracted from different plant objects and *nested*-PCR conditions were optimized.

The results of molecular detection of pathogenic fungi of genus *Fusarium* in plant material are shown in Figure 3.

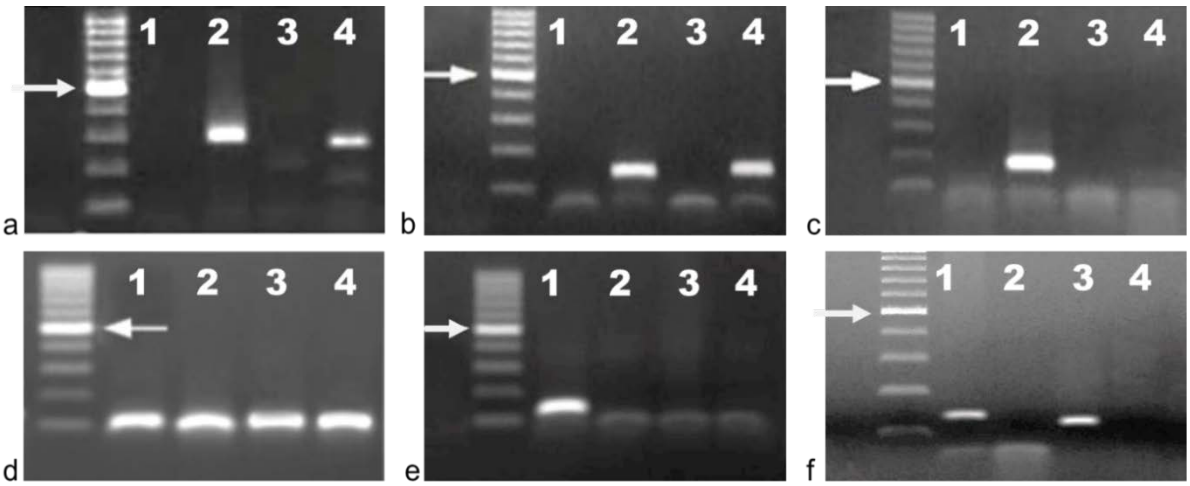

Figure 3. Electropherogram of the amplicons obtained in the second round of *nested*-PCR on total DNA extracted from plant material using primer pairs to *Fusarium* spp.
a) *Fusarium oxysporum* (line 2), *Fusarium solani* (line 4), b) *Fusarium avenaceum* (lines 2, 4), c) *Fusarium verticillioides* (line 2), d) *Fusarium equiseti* (lines 1-4), e) *Fusarium nivale* (line 1), f) *Fusarium sporotrichioides* (line 1), *Fusarium proliferatum* (line 3).
The arrow points at a 500 bp marker fragment.

The positive signals in the second round of *nested*-PCR for *Fusarium spp*. are marked with the presence of amplicons of following length: *Fusarium oxysporum* – 292 bp (Figure 3a, line 2), *Fusarium solani* – 275 bp (Figure 3a, line 4), *Fusarium avenaceum* - 140 bp (Figure 3b, lines 2, 4), *Fusarium verticillioides* - 160 bp (Figure 3c, line 2), *Fusarium equiseti* – 104 bp (Figure 3d, lines 1-4), *Fusarium nivale* – 127 bp (Figure 3e, line 1), *Fusarium sporotrichioides* – 135 bp (Figure 3f, line 1), *Fusarium proliferatum* – 123 bp (Figure 3f, line 3). The sizes of specific amplicons obtained by *nested*-PCR correspond to the parameters calculated using the BLAST software.

The results of testing primers for the identification of *Penicillium* spp. and *Aspergillus* spp. in plant material are presented in Figure 4. The presence of specific amplicons is marked of following length: *Penicillium citrinum* – 248 bp (Figure 4a, lines 1-4), *Penicillium chrysogenum* – 174 bp (Figure 4b, lines 1, 4), *Penicillium expansum* – 251 bp (Figure 4c, line 3), *Aspergillus flavus* – 144 bp (Figure 4d, lines 1, 3). The length of all fragments obtained as a result of amplification also corresponds to the parameters specified in the BLAST analysis.

It should be noted that the melting temperature of all primers is close to 60 °C and the length of the amplification products does not exceed 300 bp, which allows them to be used under the same PCR conditions. This set of primers was used for screening some crops to determine the qualitative composition of pathogenic fungi that affect plants.

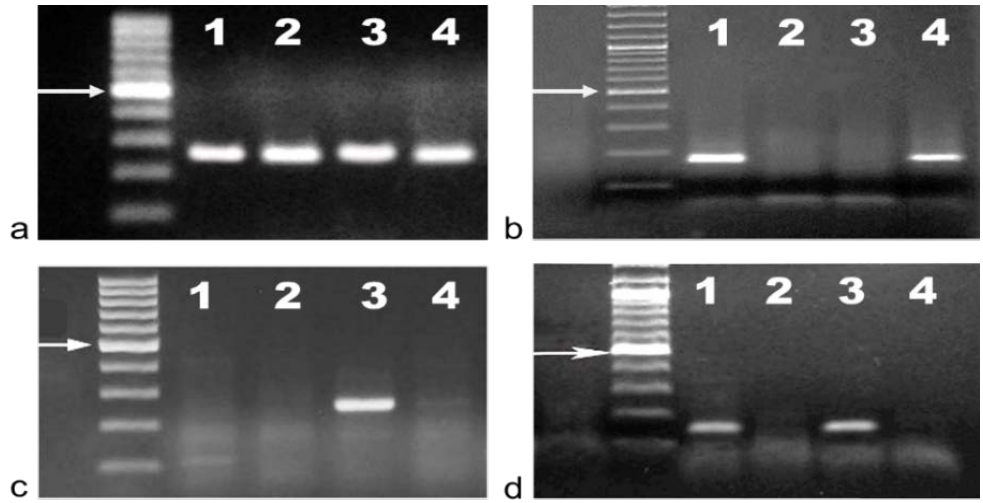

Figure 4. Electropherogram of the *nested-PCR* results obtained on total DNA extracted from plant material using primer pairs to *Penicillium* spp. and *Aspergillus* spp.: a) *Penicillium citrinum* (lines 1-4), b) *Penicillium chrysogenum* (lines 1, 4), c) *Penicillium expansum* (line 3), d) *Aspergillus flavus* (lines 1, 3). The arrow points at a 500 bp marker fragment.

3.3. Study of phytopathogenic load of seeds at different storage periods

Seed storage is associated with a number of problems. The main negative impact is caused by pathogenic microflora. Fungal infections affect stored seeds, reducing their viability and, as a result, leading to yield losses. The presence of minor amounts of pathogens in tissues or on the surface of seeds can cause mold growth when temperature and humidity fluctuations exceed admissible levels during storage. Propagation of pathogenic soil-born fungi in seeds cause their decay, necrosis, suppression of germination. Infections transmitted via seeds provoke the development of systemic plant diseases and a decrease in yield quantity and quality [14]. To prevent seed spoilage during storage, it is necessary to monitor their phytosanitary condition before preservation and during storage.

The results of molecular detection of fungal pathogens of *Fusarium, Penicillium* and *Aspergillus* genera in seeds of common bean, bell pepper and eggplant are presented in Figure 5.

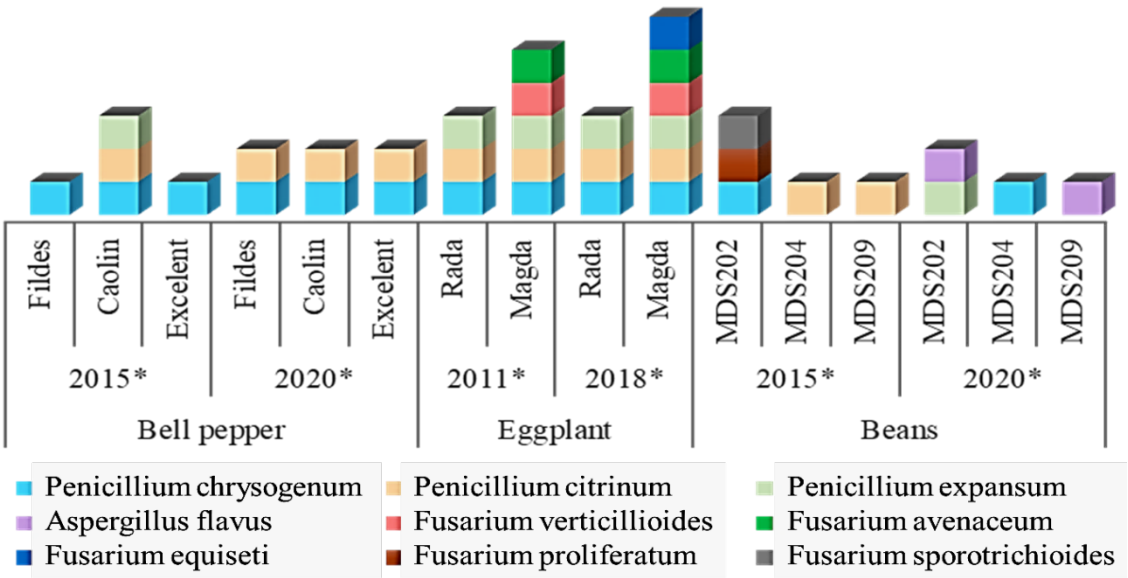

Figure 5. Species composition of pathogenic fungi in seeds of different storage periods. * - year of seeds preservation.

Fungi of the genus *Penicillium* were the predominant pathogens for all plant genotypes. *Penicillium chrysogenum* and *Penicillium citrinum* were the most detected in the seeds of eggplant and bell pepper. *Penicillium expansum* was found once in beans (MDS202, 2020 harvest) and bell pepper (Caolin, 2015). All eggplant varieties from 2011 and 2018 years of harvest were infected by *Penicillium chrysogenum, Penicillium expansum* and *Penicillium citrinum,* including bell pepper variety Caolin (2015 harvest). In beans, *Penicillium chrysogenum* (MDS204, 2020 harvest) and *Penicillium citrinum* (MDS204, MDS209, 2015 harvest) were detected. *Aspergillus flavus* was found twice in beans MDS202 and MDS209 (2020 year of seeds preservation).

Fungi of the genus *Penicillium* most often affect seeds and fruits of various crops in the post-harvest period, especially during long-term storage. During plant ontogenesis, *Penicillium* spp. and *Aspergillus* spp. behave predominantly as saprotrophs. Some species have been shown to act as pathogen antagonists by secreting antimicrobial active substances. Atoxigenic *Penicillium* and *Aspergillus* species can exhibit antifungal activity against some phytopathogenic fungi and suppressed their penetration into the host plant [15]. There are known species that are promote plant growth and resistance (PGPF - plant growth promotes fungi) and are used as biofertilizers [16,17]. However, their pathogenic potential manifests itself during harvest storage. They cause spoilage of fruits, their presence in seeds can lead to a significant decrease in germination, weakening of resistance to other factors, and even death [18]. There is a danger of the spread of pathogens to new areas when moving across international borders for trade or research purposes, as a consequence – their acclimatization, the emergence of new strains or physiological races [19]. Some species manifest phytotoxigenic activity that inhibit seed germination and plant development. Seed and fruit rots caused by filamentous *Penicillium* spp. fungi are hazardous storage diseases as they can cause huge yield losses and yield contamination with secondary metabolites called mycotoxins – a case of primary importance [20]. Therefore, control of these pathogens during crop storage is very important.

Molecular analysis demonstrated a low incidence of *Fusarium* species in studied seeds. They are absent in the bell pepper, and were detected in only one common bean genotype – MDS202 (2015 harvest). In the DNA sample of this genotype*, Fusarium proliferatum* and *Fusarium sporotrichioides* were identified. The eggplant variety 'Magda' showed increased sensitivity to *Fusarium* spp. pathogens. Thus, *Fusarium verticillioides* and *Fusarium avenaceum* were detected in the seeds of this genotype preserved in 2011 and 2018. *Fusarium equiseti* was found in the seeds of 2018 harvest.

Fusarium not only causes direct crop losses, but also reduces seed germination to the point of complete loss. Some common species of the genus *Fusarium* produce toxic metabolites – mycotoxins, the presence of which in seeds makes them unsuitable for food and feed purposes [21,22]. Therefore, monitoring of these pathogens in agricultural products is very important.

3.4. Monitoring of fungal infections during plant ontogenesis

The development of some fungal infections in plants during vegetative season was assessed: in seeds before planting, at the phase of 4-5 leaves, at the flowering phase, at the phase of fruit formation and growth, and in fresh seeds. The results of molecular analysis of eggplant genotypes during ontogenesis using primers for detection of *Fusarium* spp. and *Penicillium* spp. are presented in Figure 6.

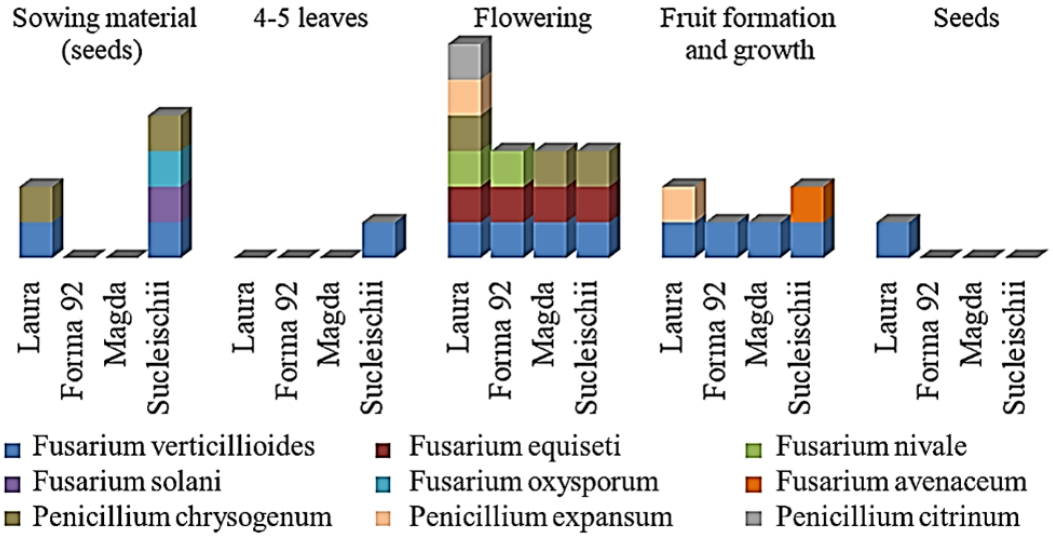

Figure 6. Distribution of *Fusarium* spp. and *Penicillium* spp. during eggplant ontogenesis.

PCR analysis revealed that the sowing material (seeds of the 2020 harvest) of two genotypes, 'Laura' and 'Sucleischii', was infected with *Fusarium verticillioides* and *Penicillium chrysogenum*, in addition, *Fusarium oxysporum* and *Fusarium solani* were detected in 'Sucleischii'. None of the pathogens were identified in the genotypes 'Magda' and 'Forma 92'. Molecular detection showed that plants are most prone to pathogenic fungi during the flowering phase. Eggplant genotypes at the phase of 4-5 leaves and "new" seeds show less vulnerability to pathogens.

The most cases of infection were noted in the 'Laura' variety during its ontogenesis. The following species of fungi were detected in this genotype: *Fusarium verticillioides, Fusarium equiseti, Fusarium nivale, Penicillium chrysogenum, Penicillium expansum, Penicillium citrinum*. 'Sucleischii' also showed increased susceptibility to *Fusarium* spp. and *Penicillium* spp. In this genotype *Fusarium verticillioides, Fusarium oxysporum, Fusarium solani, Fusarium equiseti, Fusarium avenaceum* and *Penicillium chrysogenum* were found throughout all development phases, with the exception of "new" seeds. The highest number of positive signals during vegetative season was observed for *Fusarium verticillioides*. The PCR analysis showed that fresh-harvested seeds are free of the listed pathogens, with the exception of a single incidence in the 'Laura' variety. Therefore, the eggplant seeds of this growing season are of good quality and can be used both for further reproduction and for long-term storage.

The presence of *Fusarium verticillioides, Fusarium equiseti, Fusarium proliferatum, Fusarium sporotrichioides, Penicillium expansum*, *Aspergillus flavus* in studied seeds and plants should be closely monitored, since these species of fungi belong to the group of mycotoxin producers that are dangerous to human and livestock health. This is especially relevant to agricultural products intended for storage.

Plant diseases pose a serious threat to agricultural production and food security. Factors such as climate change, changes in agricultural practices, loss of resistant varieties and loss of biodiversity provoke the risk of plant disease outbreaks. This situation not only directly leads to reduced yields, but also reduces the quality and value of agricultural products and increases agricultural costs. So, monitoring of plant diseases at local levels is necessary to improve global food security. Identification of genotypes with reduced sensitivity to fungal pathogens is of interest in terms of their further use to study the relationship between their genetic characteristics and diseases.

4. Conclusions

The efficiency of molecular technique for the effective species-specific detection of pathogenic fungi that can affect agricultural crops was demonstrated.

Molecular identification of *Fusarium spp., Penicillium spp*. and *Aspergillus spp*. in seeds of vegetable crops of different storage periods was carried out. Fungi of the genus *Penicillium* were the predominant pathogens in all plant genotypes. *Penicillium chrysogenum, Penicillium citrinum* and *Penicillium expansum* were the most detected in eggplant and bell pepper seeds regardless of the year of seed preservation. It was found that the studied eggplant seeds are the most vulnerable to pathogens of the genus *Fusarium*. Molecular diagnostic assessment of the phytosanitary status of seeds is necessary to ensure effective plant protection from a complex of seed-borne pathogens. Thus, it is not advisable to use infected seeds for long-term storage due to the risk of accumulation of metabolic products of pathogenic fungi - mycotoxins, which greatly reduces their quality.

The development of the fungal infection in some eggplant genotypes during ontogenesis was studied. Based on *nested*-PCR assay, predominant fungal pathogen in the tested plants was *Fusarium verticillioides,* which is one of the important producers of the mycotoxins. As a result of molecular analysis, studied eggplant genotypes showed varying susceptibility to fungal infections. The genotype 'Laura' demonstrated the greatest vulnerability to *Fusarium spp*. and *Penicillium spp*., the genotypes 'Magda' and 'Forma 92' showed the least susceptibility.

The species composition of *Fusarium, Penicillium* and *Aspergillus* genera affecting some vegetable crops plantings in the experimental plots of the IGPPP was shown.

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Conflicts of Interest: The author declares no conflicts of interest.

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