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ISOLATION AND IDENTIFICATION OF FUNGAL DISEASES OF WHEAT AND BARLEY TRANSMITTED BY SEEDS

Seeds are one of the crucial resources for increasing crop productivity. Improving seed quality can significantly increase crop yield potential. However, seeds are an effective tool for introducing plant pathogens into a new area and also ensuring their survival from one harvest season to the next. The microflora carried by the seeds is a significant destroyer of grains; storage makes them unfit for consumption due to a decrease in their nutritional value and often due to the formation of mycotoxins. Pathogenic microorganisms carried by seeds cause rotting of seeds during germination and death of seedlings, which leads to deterioration of the condition of crops, reduced plant growth and crop productivity. Seed-borne pathogens associated with seeds externally or internally can cause seed rot, reduced or destroyed germination, and damage to seedlings leading to the development of disease at later stages. Infected seeds play a significant role in the occurrence of economically important plant diseases in the field, leading to lower agricultural yields. The aim of this study was to isolate and detect seed-borne fungal microflora from wheat (Triticum aestivum L.) and barley (Hordeum sativum L.) seeds widely grown in Kazakhstan.

Key words: seed quality, wheat, barley, phytopathogens, seed-borne fungi.

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ИЗОЛЯЦИЯ И ИДЕНТИФИКАЦИЯ ВОЗБУДИТЕЛЕЙ ГРИБНЫХ БОЛЕЗНЕЙ ПШЕНИЦЫ И ЯЧМЕНЯ ПЕРЕДАЮЩИЕСЯ СЕМЕНАМИ

Семена являются одним из важнейших ресурсов для повышения урожайности сельскохозяйственных культур. Улучшение качества семян может значительно повысить потенциальную урожайность сельскохозяйственных культур. Однако семена являются эффективным инструментом для интродукции патогенов растений на новую территорию, а также для обеспечения их выживания от одного сезона сбора урожая к следующему. Микрофлора, переносимая семенами, является значительным разрушителем зерна. Во время хранение делает их непригодными к употреблению из-за снижения их питательной ценности и часто из-за образования микотоксинов. Патогенные микроорганизмы, переносимые семенами, вызывают гниение семян во время прорастания и гибель всходов, что приводит к ухудшению состояния посевов, снижению роста растений и урожайности сельскохозяйственных культур. Переносимые семенами патогены, связанные с семенами извне или изнутри, могут вызывать загнивание семян, снижение или полное уничтожение всхожести и повреждение всходов, приводящее к развитию болезней на более поздних стадиях. Зараженные семена играют значительную роль в возникновении экономически важных болезней растений в полевых условиях, приводящих к снижению урожайности сельскохозяйственных культур. Целью данного исследования было выделение и идентификация грибной микрофлоры, переносимой семенами из семян пшеницы (Triticum aestivum L.) и ячменя (Hordeum sativum L.) широко выращиваемых в Казахстане.

Ключевые слова: качество семян, пшеница, ячмень, фитопатогены, грибы, переносимые семенами.

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ҮРӨН АРКЫЛУУ ЖУГУУЧУ БУУДАЙ ЖАНА АРПА КОЗУ КАРЫН ООРУЛАРЫНЫН КОЗГОГУЧТАРЫН ИЗОЛЯЦИЯЛОО ЖАНА ИДЕНТИФИКАЦИЯЛОО

Уруктар өсүмдүктөрдүн түшүмдүүлүгүн жогорулатуу үчүн эң маанилүү ресурстардын бири болуп саналат. Үрөндүн сапатын жакшыртуу өсүмдүктөрдүн потенциалдуу түшүмдүүлүгүн кескин жогорулатат. Бирок, уруктар өсүмдүктөрдүн козгогучтарын жаңы аймакка киргизүү, ошондой эле алардын бир түшүм жыйноо мезгилинен кийинки мезгилге чейин жашоосун камсыз кылуу үчүн натыйжалуу курал болуп саналат. Урук ташыган микрофлоралар данды Олуттуу бузуучу болуп саналат. Учурунда сактоо аш болумдуулугунун төмөндөшүнөн жана көбүнчө микотоксиндердин пайда болушунан улам аларды жараксыз кылат. Үрөн ташыган

патогендик микроорганизмдер үрөндүн өнүп чыгышы учурунда чирип, көчөттөрдүн өлүшүнө алып келет, бул эгиндин начарлашына, өсүмдүктөрдүн өсүшүнүн жана түшүмдүүлүгүнүн төмөндөшүнө алып келет. Сырттан же ичинен уруктар менен байланышкан үрөн аркылуу жугуучу патогендер үрөндүн чиришине, өнүү агымынын азайышына же толугу менен жок болушуна жана кийинки этаптарда оорулардын өнүгүшүнө алып келиши мүмкүн. Ылаңдаган уруктар талаада өсүмдүктөрдүн экономикалык жактан маанилүү ооруларынын пайда болушунда чоң роль ойнойт, натыйжада айыл чарба өсүмдүктөрүнүн түшүмдүүлүгү төмөндөйт. Бул изилдөөнүн максаты Казакстанда кеңири өстүрүлгөн буудайдын (Ощ.) жана арпанын (Ощ.) уруктарынан ташылган козу карын микрофлорасын бөлүп алуу жана идентификациялоо болгон.

Ачкыч сөздөр: үрөндүн сапаты, буудай, арпа, фитопатогендик, уруктуу козу карындар.

Introduction. Seeds are the most important primary product for crop production. Many plant pathogens are seed-borne, which can cause huge crop losses. The rise in the production and sale of organic seeds has increased the focus on the quality of organic seeds. Also, he drew attention to the problems of infection with diseases transmitted through seeds. Seed-borne diseases are pathogenic microorganisms such as bacteria, fungi or viruses that live on or inside seeds and can potentially spread disease to a subsequent crop. Of these, the most important is fungal contamination [1]. Plant diseases annually damage the yield of major crops by up to 10-15 per cent. From 70 to 80 per cent of it is caused by pathogenic agents of fungal diseases [2]. Seed-borne fungi are one of the most important biotic constraints on seed production worldwide. They are responsible for both the death of grains before and after emergence, affect the viability of seedlings and thus cause some reduction in germination, as well as changes in plant morphology [3]

The fungi infection can be spread through direct or indirect contact. Direct effects on seed quality may be due to the growth and branching of the fungus throughout the grain and the formation of metabolites that can alter the composition of the grain or render it unfit for human or animal consumption. Indirect effects are related to the reduction in yields that is associated with pollution. Infected seeds play a significant role in the spread of an economically important plant disease in the field, leading to severe reductions in crop yields. Contaminated seeds are also of lower quality, resulting in lower market value, poor germination and establishment in fields [1].

Losses caused by seed fungi can occur during seed development, storage, or germination. If the temperature and humidity of the seeds are not maintained properly during storage, cool, humid conditions during sowing increase the growth of these fungi and their subsequent damage [1,3].

However, seed-borne diseases are not ubiquitous in all crops, and special attention should be given first to specific diseases known to be seed-borne that pose a risk in a particular growing region. The risk of contracting seed-borne diseases varies greatly by crop, disease and location. Many diseases will only become a problem if grown in a region or environment that is favorable to the disease. Typically, diseases present in seeds can also be transmitted by soil or air, and the ultimate fate of the crop may depend on the resistance of varieties and agricultural practices [1,6].

Wheat (*Triticum aestivum* L.) and barley (*Hordeum sativum* L.) are staple foods in many regions of the world and are currently widely cultivated crops. Due to the increase in the world population, it is very important to increase wheat production. It is estimated that almost 50% more wheat production will be required in 2050 due to an increase in population. Increasing wheat yield is the biggest challenge for researchers. It faces several biotic and abiotic limiting factors. However, the main threat to wheat is the large number of pathogens

affected by fungi, which leads to massive and devastating crop losses. In 2019, it was estimated that nearly 22% of wheat crop losses were caused by disease. Over time, these percentages will increase due to mutations and diversity of virulent strains [4].

The wheat crop is susceptible to a number of seed-borne pathogens, which could significantly reduce its global production [5]. The seed fungal complex, including the genera *Tilletia, Ustilago, Bipolaris, Fusarium, Alternaria, Aspergillus* and *Penicillium,* are the most commonly seed-borne wheat fungi worldwide. (6) In recent years, spotting caused by *Bipolaris sorokiniana* (Sacc.) syn. *Drechslera sorokiniana* (Sacc.) has become a serious problem in wheat production in warmer, wetter regions of the world. During the last two decades, there have been significant economic losses in wheat production due to blight. In addition to blight, this fungus is also the causative agent of other diseases such as common root rot, seedling damage and seed rot of wheat [6].

Some seed-borne fungal pathogens of barley include *Alternaria alternata, Aspergillus candidus, A. flavus, Curvularia lunata, Drechslera halodes, Fusarium moniliforme* [7]. The most common fungal species carried by barley seeds is Alternaria spp. widespread and includes plant pathogenic and saprophytic species that can damage crops in the field and cause post-harvest rot. The disease can become a major issue for barley in areas where heavy rainfall occurs during the early stages of grain development [8].

To control plant diseases, the main stage is the use not infected with phytopathogens of seeds. To determine seed status and improve seed germination potential, it is essential to research seed pathogens, which ultimately lead to increased crop yields. The purpose of the study was to identify fungal microflora associated with wheat and barley seeds available on the market. The results would provide empirical information on the most contaminated varieties and possible action against pathogens.

Materials and research methods. The current research was carried out in the laboratories of the Testing Center for Phytosanitary Laboratory Analysis, Kazakh Research Institute of Plant Protection and Quarantine within the framework of the scientific and technical program BR10764960 “Development and improvement of integrated systems for the protection of fruit, vegetable, grain, fodder, legumes and plant quarantine.” When wheat and barley seeds were analyzed we used government standard 12044-93 [9]. Isolation, propagation and identification of fungi were carried out using standard laboratory protocols in accordance with ISTA. [10]**.**

The Institute's collection includes five varieties of wheat seeds and barley that were tested. Standard 400 g seed samples were stored in paper bags and marked.

The contamination of seeds was determined by sprouting in a wet chamber and on growth media. The collected seeds were subjected to surface sterilization with 96% alcohol for 1-2 minutes, then rinsed three times with sterile water and dried between sheets of sterile filter paper.

Seed analysis in a wet chamber is the most popular and often used to detect multiple fungi that can cause mycelium growth and fruiting during incubation. This method is most commonly used to detect seed-borne diseases. All the collected seeds were placed in petri dishes, each of which was covered with two layers of water-soaked filter paper. The seeds on the cup were incubated for seven days at a temperature of 20-22°C. To stimulate the formation of conidienes and conidium to identify pathogens of some fungi, there was a need for 12 h alternating light and darkness when sprouting seeds in cups. Microflora was examined under a microscope to detect the presence of infection inside the seeds [9.16].

Seed analysis on growth media is used to identify and detect micro-organisms. In many diseases, the fungal fungus is kept inside the seeds, and in appearance, these seeds are difficult to distinguish from healthy. This method is based on stimulating the development and growth of external and internal infection in infected seeds. For the excretion of mycophlors, potato dextrose agar (PDA) was used. Modified PDA having (20 g of potato

extract, 20 g of agar and 20 g of dextrose) prepared according to the instructions. The Medium was sterilized in the autoclave and poured into glass Petri cups of 20 ml diameter of 9 cm. From the average sample take 4 samples of 50 pcs. in each and place them in sterile utensils with a nourishing medium. Seeds in each cup were seeded with sterile tongs. All Petri cups were held for 7 days at 20°C during 12-hour alternate cycles of near-ultraviolet light and dark. All sliding panes were examined under stereoscopic microscope to observe mycofluoric within the seeds [9.17]

Molecular genetic research of fungi identification consists of the following stages: isolation of common DNA, amplification by PCR, comparative analysis (identification) of the PCR product (sequencing), and data obtained from sequencing with data from the database.

From pure fungal cultures, DNA extraction was carried out with a reagent" «Проба-ГС» (ООО «НПО ДНК-Технология», Russia according to manufacturer's protocol.

The method of electrophoresis in agarose gel was used to test isolated DNA samples and amplified PCR products. The DNA concentration in the resulting preparation was determined visually by the glow intensity of a 10 µl sample in under ultraviolet light in a 1% agarose gel with the addition of 2 µl bromide ethidia. Electrophoresis was carried out at a voltage of 100 V for 1 hour. The Generuler (Thermo Scientific, US) marker was used as a benchmark. The results of electrophoresis were analyzed using the gel documentation system transilluminator Quantum-ST 5 (Vilbert Lourmat) France.

PCR analysis was carried out using its 1 (5'TCC GTA GGT GAA CCT GCG G '3) and reverse its 4 (5'tcc TCC GCT TAT TGA TAT GC' 3) primers [19]. In the mixture of reagents required for the PCR reaction (25 µL), there were 4 µL of HF buffer (Thermo Scientfic), 0.5 µL of deoxyribonucleoside triphosphate (dntp) mixture, 0.3 µL of each of the primers mixture, DNA polymerase enzyme Phusion High-Fidelity DNA Polymerase (Thermo scientific) 0.2 µL and 2 µL of DNA. The reaction was carried out in the Thermocycle SimpliAmp Thermo Cycler (Life Technologies Corporation) according to the PCR program: initial denaturation - 98°C, temperature 30 sec, 98°C – 10 sec, 60°- 20 sec and final extension 72°C-30 for 5 min at 72°C.

Purification of PCR fragments from foreign impurities was carried out using a set of reagents "ExoSap IT^{TM}".

Determination of the direct sequence of nucleotide sequences of the its region was carried out in the Sanger sequencer (Applied Biosystems, Genetic Analyzer 3500).

The nucleotide similarity of the sequences was evaluated using BLAST in the open genetic database NCBI and Bold System. The sequences were aligned by using the MEGA version. 7.0. [20-22].

Results and their discussion. Almost all samples of wheat seeds and barley are dominated by fungi of Alternaria sp. Fusarium spp. is a pathogen of fusarium and root rot. Five varieties of wheat have also been separated from the fungi of the genera *Bipolaris sp.* causative agent of root rot *and Penicillium sp. Epicoccum sp*. The most commonly isolated species were *Alternaria sp.* The seeds of all studied barley varieties also contained *Culvularia sp*. The most commonly excreted fungi included *Fusarium spp*. (Table 1).

Table 1

Fungal microflora from wheat and barley varieties

№						
		1 grade	grade	grade	grade	grade
	sp.					
	sp.		-	-		
	sp.		-	-	-	
	sp.	-	-	-		-

	Fusarium sp.					-
From barley seed						
	sp.					
	Culvularia sp.	-	-	-	-	
	Fusarium spp.					

On barley seeds, a high degree of seed infection by root rot pathogens is revealed by phytoexamination of seeds (dominant: *Fusarium* up to 28%, *Helminthosporium* and *Alternaria* up to 26%, bacteriosis up to 10%, seed molding up to 5%). Protection against root rot: mandatory compliance with crop rotation, high-quality destruction of plant residues, compliance with the norm for applying nitrogen fertilizers, growing resistant varieties, mandatory treatment of seeds before sowing (Figure 1).

Analysis of nucleotide sequences. Raw sequences were analyzed by Bio-Edit software After that, the end fragments (nucleotide sequences of primers, fragments having a low quality index) were removed. The resulting sequences were identified in GeneBank using the BLAST algorithm.

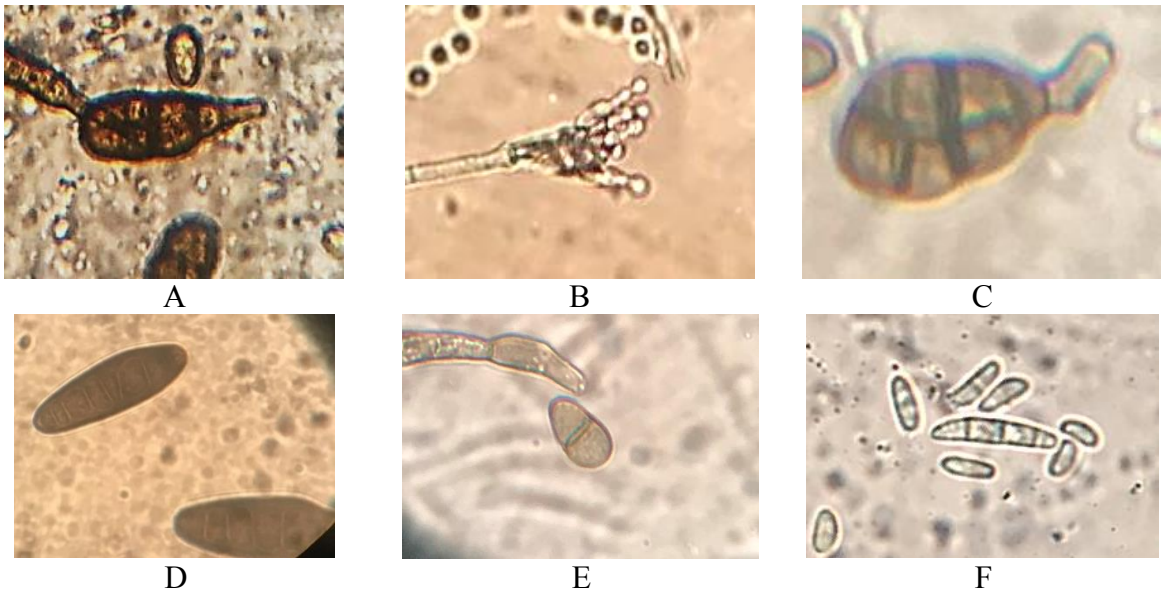

Figure 1. Fungal microflora isolated from different varieties of wheat and barley: *Alternaria* (A), *Penicillium* (B), *Epicoccum* (C), *Bipolaris* (D), *Culvularia* (E) and *Fusarium* (F)

Additionally, phylogenetic trees with sequences deposited in the GeneBank international database were constructed with Mega 7 software which was used to build phylogenetic trees. The ClustalW algorithm was used to align nucleotide sequencs, the tree construction was carried out using the Neiighbor-Joining NJ method [18, 20].

Phylogenetic tree of several species of fungi of the genus *Fusarium*, constructed based on the alignment of nucleotide sequences of the internal transcribed spacer (ITS) region. The figure shows that the sample sequence is located on one branch of *Fusarium proliferatum*, and the H3 sequence of the sample lay down with such species of *Fusarium* as *equiceti*, *incarnatum*.When constructing a phylogenetic tree, the sequence of sample H1 was combined into one phylogenetic branch with *Fusarium proliferatum*. Taking into account the maximum percentage of coincidence of the analyzed sequence in the international database using the BLAST algorithm, as well as the results of phylogenetic analysis, it was established that samples H1 belong to *Fusarium proliferatum*; however, for sample H3, additional research methods must be used to differentiate the species (Figure 2).

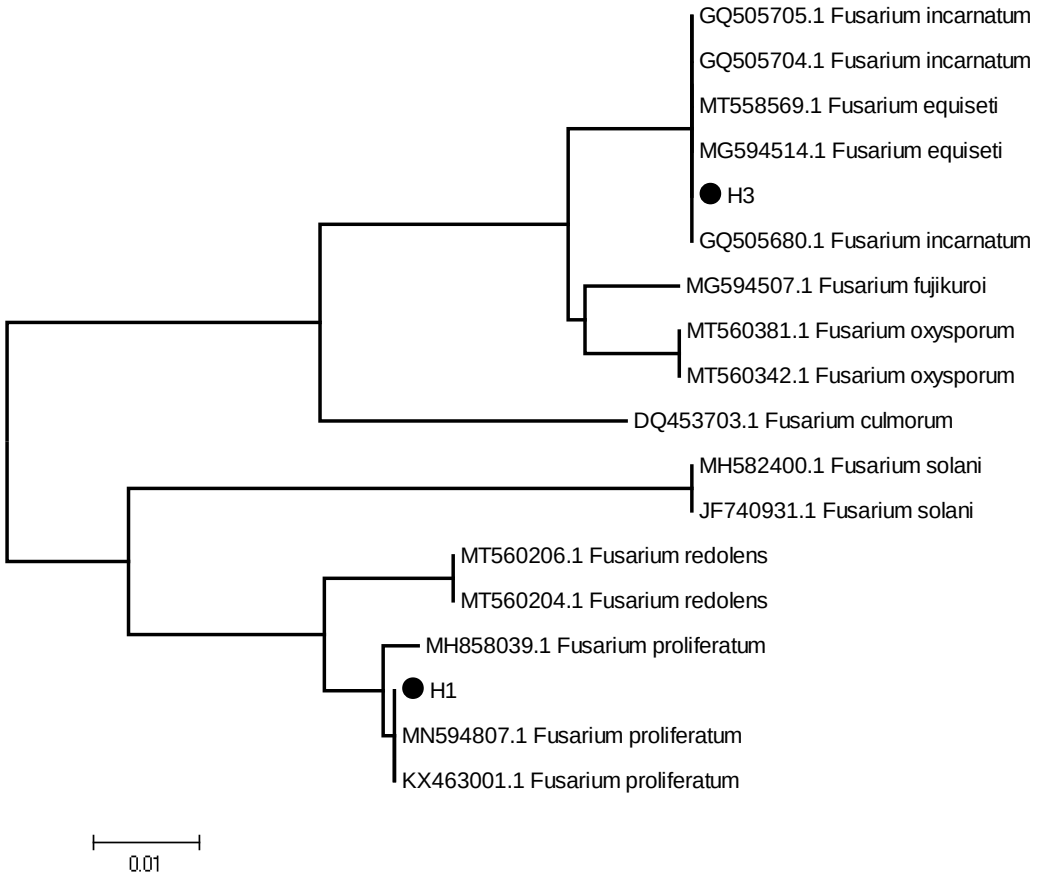

Figure 2. Phylogenetic analysis of *Fusarium*-based ITS region

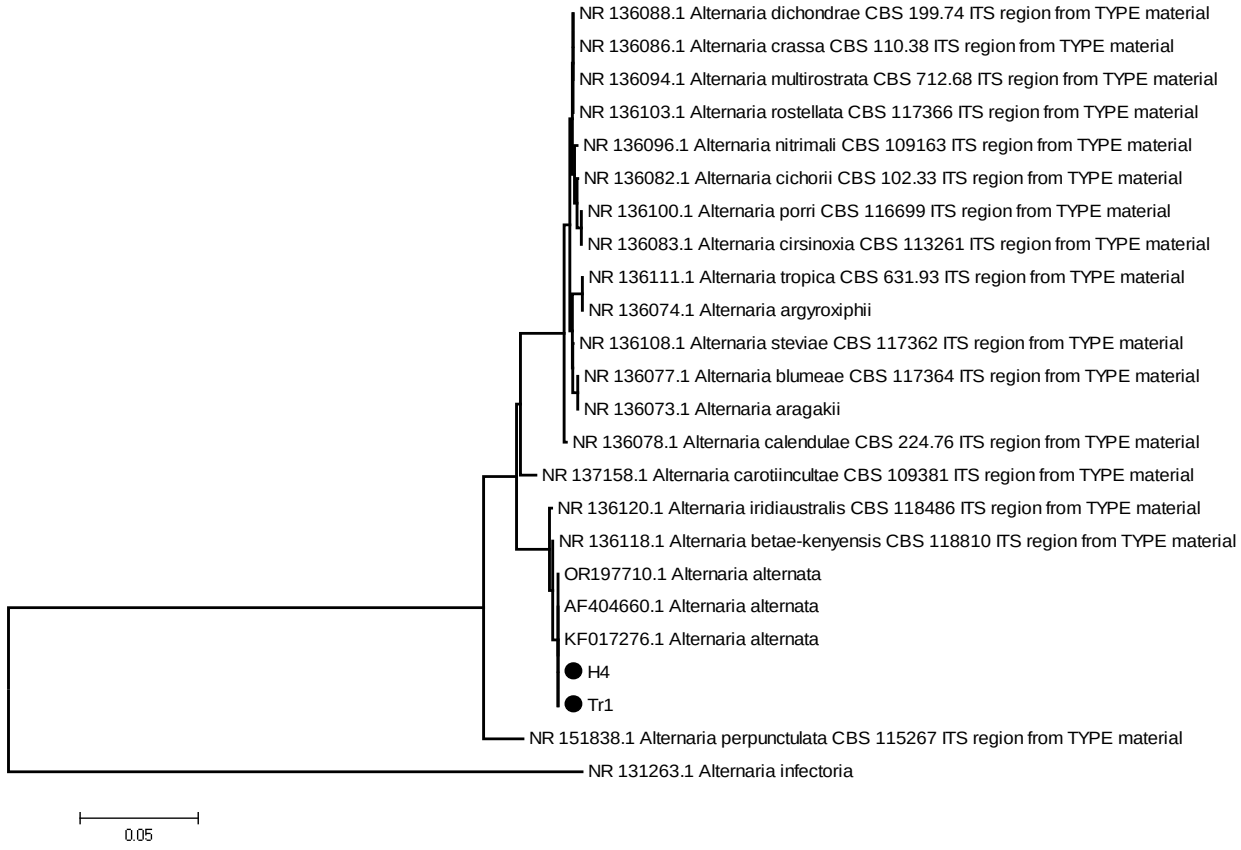

Figure 3. Phylogenetic analysis of *Alternaria*-based ITS region

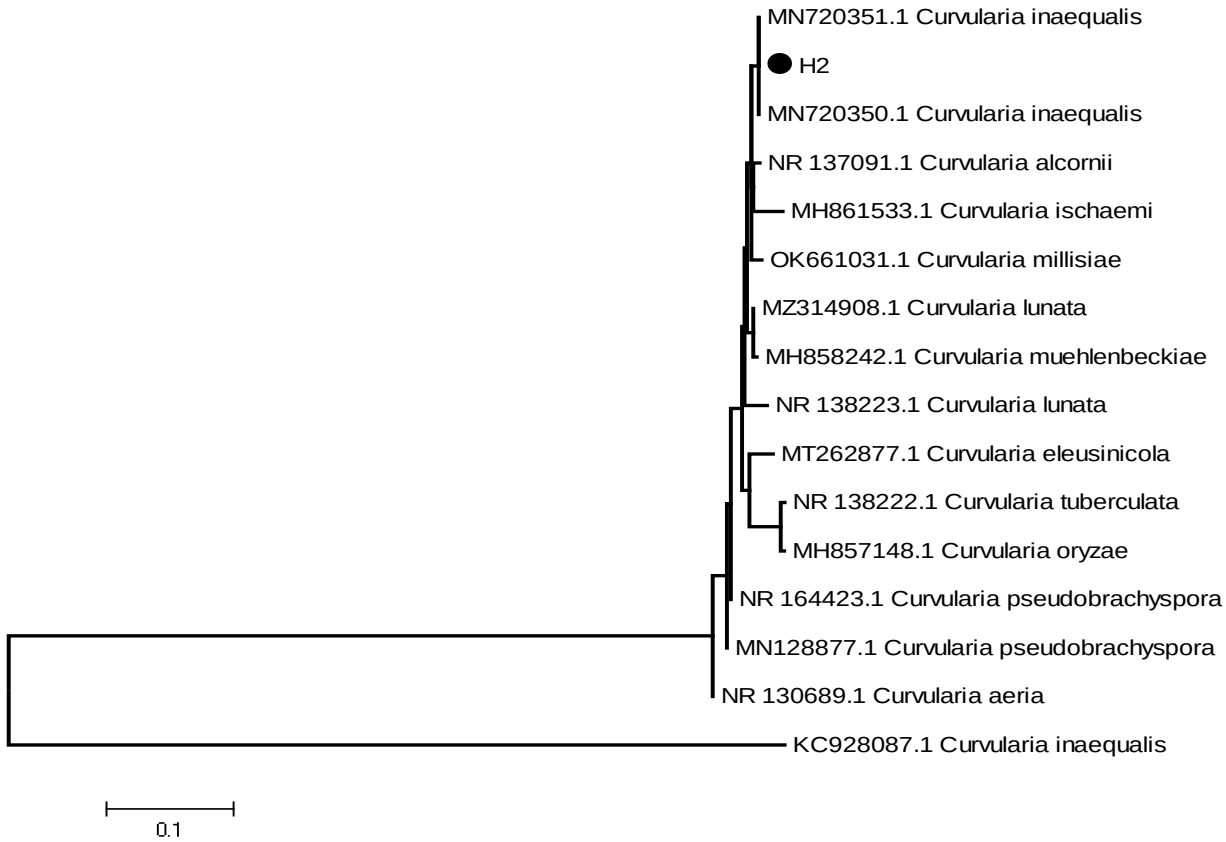

Figure 4. Phylogenetic analysis of *Culvularia*-based ITS region

Phylogenetic tree of several species of fungi of the genus *Alternaria,* constructed based on alignment of nucleotide sequences of the internal transcribed spacer (ITS) region. The figure shows that the sequence of H4 and Tr1 samples is located on the same branch with *Alternaria alternata*. When constructing a phylogenetic tree, the sequences of H4 and Tr1 samples were combined into one phylogenetic branch with *Alternaria alternata*. Taking into account the maximum percentage of coincidence of the analyzed sequence in the international database using the BLAST algorithm, as well as the results of phylogenetic analysis, it was established that samples H4 and Tr1 belong to *Alternaria alternata* (Figure 3).

Phylogenetic tree of several species of fungi of the genus *Culvularia,* constructed based on the alignment of nucleotide sequences of the internal transcribed spacer (ITS) region. The phylogenetic tree was constructed using the Mega version 7 program. The figure shows that the H2 sequence of the sample is located on one branch of *Culvularia.* When constructing the phylogenetic tree sequence H2, the sample was combined into one phylogenetic branch with *Curvularia inaequalis.* Taking into account the maximum percentage of coincidence of the analyzed sequence in the international database using the BLAST algorithm, as well as the results of phylogenetic analysis, it was established that sample H2 belongs to *Curvularia inaequalis* (Figure 4).

Phylogenetic tree of several species of fungi of the genus *Epicoccum,* built on the basis of alignment of nucleotide sequences of the internal transcribed spacer (ITS) region. A phylogenetic tree was constructed using the Mega version 7 program. The figure shows that the sequence of sample Tr2 is combined with different *Epiccocum* species. Given the high identity of this genus, additional analytical methods are required for reliable identification (Figure 5).

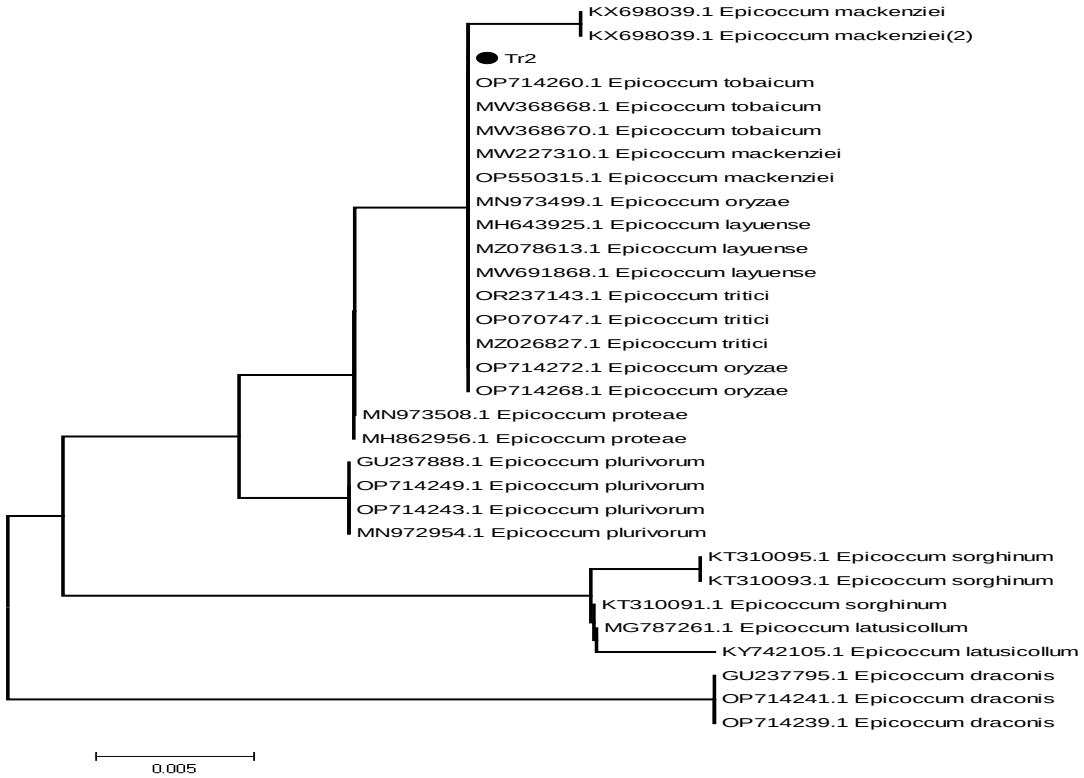

Figure 5. Phylogenetic analysis of *Epicoccum*-based ITS region

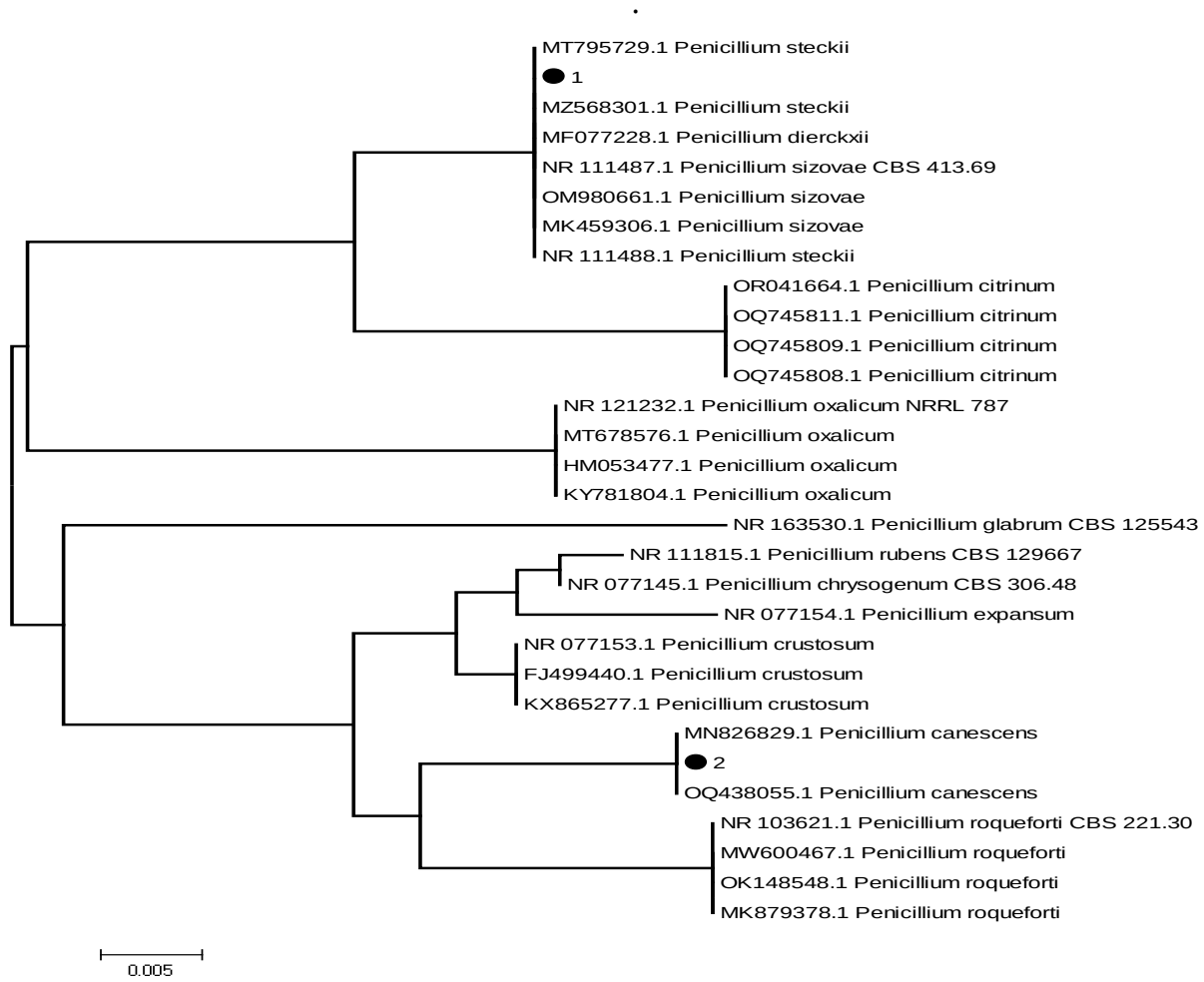

Figure 6. Phylogenetic analysis of *Penicillium* -based ITS region

Phylogenetic tree of several species of fungi of the genus *Penicillium*, constructed based on the alignment of nucleotide sequences of the internal transcribed spacer (ITS) region. Phylogenetic trees were constructed using the Mega version 7 program. The figure shows that the sequence of sample 2 is located on one branch of *Penicillium canescens*, and the sequence of 1 sample lay down with the following species *Penicillium steckii, dierckxii* and *sizovae*. When constructing a phylogenetic tree, the sequence of sample 2 was combined into one phylogenetic branch with *Penicillium canescens.* Taking into account the maximum percentage of coincidence of the analyzed sequence in the international database using the BLAST algorithm, as well as the results of phylogenetic analysis, it was established that sample 2 belongs to P*enicillium canescens*; however, for sample 1, additional research methods must be used to differentiate the species (Figure 6).

The results of phytoexpertise showed that the seeds were infected with a complex of fungal microflora, when the seeds swell, will multiply intensively and can create an infectious background for molding of seeds, damage to plants by root rot, *Fusarium, Alternaria* during the growing season, as well as deteriorate the sowing quality of seeds, reduce germination energy and plant productivity. Many of the fungi found can affect seed health. Fungi of the genera *Alternaria, Fusarium* and *Penicillium* are capable of destroying from 50 to 90% of the grain crop yield. In addition, fungi of the genera *Alternaria, Penicillium* and *Fusarium* are capable of infecting harvested crops in storage, which leads to changes in the organoleptic properties of products, loss of presentation and rotting. Most fungi of these genera are producers of mycotoxins—secondary metabolites that pose a serious threat to human health. Mycotoxins have varying degrees of toxicity. Therefore, their permissible concentrations in food products are strictly regulated [11].

The most commonly isolated fungus, *A. alternata*, is not considered as pathogen. Seeds affected by *Alternaria* have low germination energy and germination. During germination, deformation of the seedling, darkening of the primary roots, root collar and stem base are observed. Later, during grain ripening, blackening of the embryo is observed. The disease also affects leaves at the end of the growing season, which accompanies the development of other, more aggressive pathogens. Harmfulness directly depends on the climatic conditions under which the grain ripened and the conditions of its storage [12].

Seed-borne *B. sorokiniana* is important because the pathogen is the causative agent of common root rot [13]. The fungus causes the death of seedlings and seedlings, stunted plants, a decrease in overall and productive bushiness (25%), and root rot. Fungi cause a complex plant disease: affecting the roots, stem, leaf apparatus and ear. Yield losses can be from 10% or more, seed germination can be reduced by 40%. You should know that fungi produce mycotoxins that are dangerous to both humans and animals. Under the influence of damage to the ear by *Fusarium*, the number of grains, the weight of 1000 seeds decreases, the germination of seeds decreases, and partial death of seedlings occurs. Infection of ears with fungi of the genus *Fusarium* leads to yield losses of up to 50% [11].

Saprophytic fungi of the genera *Penicillium* and *Epicoccum* can also cause significant damage to seeds, as well as phytopathogenic fungi. In the field, these fungi develop in years with high humidity, during ripening and harvesting. When the spike is fully populated with saprotrophs, crop losses can be up to 80%, with a partial settlement of up to 30%. In addition, with the strong development of fungi, the grain can acquire toxic properties. The seeds affected by saprotrophic fungi are able to re-digest [14]

Conclusions. The molecular identification was carried out by DNA bar-coding using the ITS region sequencing. The DNA sequences were compared to those in the databases using NCBI-BLAST. Eight species were identifed using DNA bar-coding with an identical range between 97–100%. It is also proposed that DNA region sequence is one of the most important tools for the identifcation of the fungal species isolated from wheat. Hence, it has

been widely used to detect the wheat fungal community, and as an improvement of the classical identifcations.

Overall, the results of our study indicate that mixed infections caused by two or more pathogens were widespread, although these infections may have been missed by visual inspection. Fungal infections have catastrophic consequences for both human and animal health and global food security. Fungal diseases lead to losses of grain crops associated with a decrease in yield and deterioration in grain quality. These consequences lead to annual losses for producers, as well as higher prices for consumers. Improving the performance of global agricultural and related food systems includes taking stock of pathogens. This is because pathogens are widely recognized as significant barriers to regular and reliable food systems. Global efforts are focused on integrated breeding techniques to improve resistance, advanced monitoring and detection technologies, and molecular insights to find disease biomarkers to eliminate fungal infections in crops [15].

Every effort should be made to use disease-free seeds to minimize losses in quality and quantity. This study demonstrated that isolated fungal species have a significant relationship in causing diseases in cereal crops and can lead to changes in the rhizosphere. This high inoculum density and maximum fungal biomass in the seeds can serve as a significant indicator of maximum disease development. This association with increased fungal abundance in seeds may promote maximum growth of fungal saprophytes.

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ЗНАЧЕНИЕ ПРЕДВАРИТЕЛЬНОГО ОХЛАЖДЕНИЯ В ХРАНЕНИЯ ПЛОДООВОЩНОЙ ПРОДУКЦИИ

В данной статье рассмотрены вопросы хранения и переработки плодоовощной продукции. Успех хранения плодоовощной продукции зависит от того, какие условия будут созданы для хранения. Созданием оптимальных условий хранения можно повысить лежкость плодов и овощей и, наоборот, при нарушении режима хранения можно полностью потерять лежкую продукцию. Процесс предварительного охлаждения плодоовощной продукции является основным условием и залогом сохранения качества плодоовощной продукции при ее закладке на продолжительное