reaching impact on the value of yield, one can name agricultural work, cultivation conditions and agricultural technology, the most important indicator of which is the conditions of irrigation of agricultural crops. As a result of the research, a direct connection was revealed between the productivity of soybean varieties and natural-climatic and various irrigation conditions over the years of determination. Our research was carried out in field and laboratory conditions. The general productive and structural elements of soybean varieties under conditions of traditional and drip irrigation were compared. The following study was carried out in laboratory conditions using the state varietal methodology for researching agricultural crops, yield structure and biometric calculations of soybean plants. In research work, the varieties Ivushka (st), Rusiya, Bayan, Alua, Almaty, Iskra, Misula, Zara, Zhalpaksai, and Rosa were obtained. Comparative indicators of productivity and structural elements of products under the conditions of traditional and drip irrigation of the specified variety samples have been determined. The only reason why the varieties obtained as a result of the study had indicators of different degrees of productivity and structural elements was directly related to weather conditions and the conditions of traditional and drip irrigation. Under drip irrigation conditions in 2023, higher yields were obtained for the varieties Zhalpaksai - 7.5 t/ha, Rusiya - 7.3 t/ha, Alua – 5.2 t/ha; Rose – 5.0 t/ha than in 2022. While the elements of the soybean yield structure differed in the number of plant branches and pods, compatibility was noted with the traits of germination density and weight of 1000 seeds. For example, the following varieties have obtained high results in the following yield structure: germination density of Jalpaxai – 481.0 thousand hectares/ ha, Russia – 477.7 thousand / ha; number of branches per plant - 3.0 pcs., Alua – 2.9 pcs., Flat-2.9 pcs.; number of pods per plant – Jalpax – 51.2 pcs., Missoula-47.9 pcs., Alua-47.7 pcs.; number of seeds per plant – 101.0 g., Alua – 88.8 g., Rose – 87.6 g.; Weight of 1000 grains – sparks – 146.2 g., placers-145.3 g. As a result, a higher yield of variety samples was obtained under drip irrigation conditions than with traditional irrigation.

***Key words**: Soybeans, varieties, seeds, yield, structure, analysis, traditional, drip, irrigation, weight.*

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## IDENTIFICATION OF THE MAIN DISEASES OF WALNUT IN THE SOUTHERN FRUIT-GROWING ZONE OF KAZAKHSTAN

*Abstract*

Industrial cultivation of nut crops in the Republic of Kazakhstan is promoted by favourable soil and climate conditions, especially in the southern region, which makes it possible to obtain high yields

of this valuable plant in the future. Significant damage to the yield of nut crops is caused by diseases, protection of plantations from which is one of the main measures aimed at increasing their productivity. The most important issue in cultivation of nut crops is its protection from bacterial and fungal pathogens. In Kazakhstan purposeful researches on studying species diversity of diseases and pests of nut crops have not been carried out. The novelty of the conducted research is in the fact that for the first time in Kazakhstan fungal and bacterial diseases of walnut in the conditions of the southern zone of fruit growing on the basis of microbiological and molecular-genetic methods were identified.

The results of research showed that the majority of isolated pathogens are fungi, as well as several species of bacteria causing diseases of walnut. In total, 4 genera of fungi were isolated: *Alternaria, Fusarium* as causative agents of fungal diseases*; Aspergillus, Penicillium* as secondary infestation; also 2 genera of bacteria: *Pantoea, Pseudomonas* as causative agents of bacterial diseases. Molecular identification of fungal and bacterial pathogens by PCR based on genomic DNA showed that the studied walnut samples were identified as phytopathogenic fungi: *Alternaria alternata, Fusarium solani,* as well as phytopathogenic bacteria*: Pantoea agglomerans, Pseudomonas syringae, Pseudomonas oryzahabitans.* The conducted research results will allow to make up a system of protection of walnut from harmful diseases in order to increase the productivity of plantations.

***Key words:*** *Walnut, bacterial diseases, fungal diseases, pathogenicity assay, molecular identification, genomic DNA, PCR analysis*

***Introduction***

From cultivated nut-bearing plants, the most widespread and valuable plant in the world, rich in nutrients, is the walnut *Juglans regia L*. The exceptional value of the species contributes to the constant increase of its areas where this plant can grow. The area of wild and cultivated walnuts covers a huge area of Eurasia: this is the north of China and India, Central Asia, in the south of Europe and in its central part. Walnuts are cultivated in the North Caucasus, Transcaucasia, Uzbekistan and Moldova [1]. The leading producer of walnut crops is the USA. In the 2017/2018 season, the country's production share was 38% with more than 1.6 million tonnes of nut kernels. Turkey and China account for 10% and 9% of global nut production, respectively. Then stand Iran (7%) and India (5%). The rich heritage of nut crop gene pool has been explored in the western region of Azerbaijan [2].

High consumer demand in domestic and foreign markets, as well as increased interest in walnut cultivation among farms and private agricultural producers, stimulated research on introduction, adaptation to obtain quality planting material of walnut in the conditions of Kazakhstan [3]. In 2017, the "Kazakhstan Association of producers and processors of nuts and berries" was registered [4]. Annually the areas under industrial walnut orchards are expanded in Almaty and Turkestan regions, where soil and climate conditions are most suitable for growing walnuts [5].

The walnut orchard LLP "Integration - Turgen" is planted on 20 hectares of land in the village of Turgen, where the main varieties of walnut existing in the world are represented. In the nursery of LLP "Saryagash zher syyy" here are 230 local forms of walnut from all regions of southern Kazakhstan. In the State State Enterprise "Issyk State Dendrological Park" walnut grows on an area of 0.4 ha [5].

The expansion of walnut production led to the appearance of damage and lesions of trees by diseases, which can lead to the loss of yield and deterioration of its quality. Under favourable conditions for the development of diseases, the intensity of their development increases and yield losses can reach up to 60-80% [6, 7]. One of the most widespread bacterial diseases in all regions of walnut cultivation is walnut blight, caused by the bacterium *Pantoea agglomerans. Pantoea agglomerans* belongs to the *Enterobacteriaceae* family [8, 9] and can infect various hosts. In China, the bacterial pathogen, *Pantoea agglomerans*, was identified as the causal agent of apical necrosis [10], whereas fungal pathogens of the genus *Fusarium and Alternaria* have been isolated from symptomatic walnut fruits in Spain, Italy and France [11,12,13]. The first report that *Pseudomonas oryzihabitans* causes walnut leaf spot and may pose a threat to walnut cultivation was made in China

[14], and the presence of *Pseudomonas syringae pv. syringae* as a causative agent of walnut leaf spot was reported in Iran [15].

Phytopathogenic fungi and bacteria cause significant economic damage to walnut plants and require constant control. In this regard, identification of walnut diseases is an urgent task. The information obtained in this study will serve as a basis for the development of necessary methods of treatment of walnut against diseases. The objects of the study were local forms and introduced varieties of walnut.

***Methods and materials***

The research work was carried out in field and laboratory conditions. For in time detection of fungal and bacterial diseases, regular surveys of walnut plantations in the south and south-east of Kazakhstan during the growing season were carried out. Samples from different parts of plants (leaves, fruits, young shoots, bark, stems) were taken from trees with evident symptoms of disease damage for microbiological studies. During sampling, diseased parts of the plant were cut with healthy tissue [16].

*Isolation of pathogens from disease infected tissues of walnut tree*

The wet chamber method was used to stimulate mycelial growth or fungal sporulation [17].

Identification of the species composition of phytopathogenic fungi was carried out using classical microbiology methods, taking into account the shape and diameter of colonies, type of sporulation, size and shape of conidia formed on potato-glucose agar medium [18]. Laboratory studies to identify bacterial pathogens were carried out according to the generally accepted methods of M.A. Chumayevskaya and E.V. Matveeva [19]. To identify the sources of infection, leaves with symptoms of wilting and spotting were subjected to bacteriological analysis. Bacteria that corresponded in morphological structure, consistency and color to phytopathogenic bacteria were selected from bacterial colonies grown on Nutrient agar medium [20]. Transfection into pure cultures of fungal and bacterial isolates was carried out in triplicate for accuracy of results.

*Pathogenicity assay*

The pathogenicity of the bacteria was tested by the infection-infiltration method of Clement's hypersensitivity reaction on the tested leaves of indoor geranium *(Pelargonium zonala L.)* [21]. For this purpose, a daily culture of the isolate with an inoculum concentration of 109 kl/ml was used according to the turbidity standard. The bacterial suspension was injected into the intercellular space of geranium leaves using a sterile syringe. Sterile water with the same plant indicator was used as control.

*DNA isolation, amplification by polymerase chain reaction and DNA sequencing*

Bacterial genomic DNA was isolated using GeneJET Genomic DNA Purification Mini Kit (ThermoFisher, USA), and from fungal isolates using Proba-GS kit (LLC "Agrodiagnostika", Russia) according to the instructions proposed by the manufacturer of these reagents.

PCR amplification was performed with universal primers: for fungi - ITS1 5′-TCCGTAGGTGAACCTGCGG -3′ ITS4 5′-TCCTCCTCCGCTTATTGATATATATGC -3′; for bacteria - 704 F 5′-GTAGCGGTGTGAAATGCGTAGA -3′ 1495 R 5′-CTACGGCTACGCTACCTTGTTACGA-3′ including pre-denaturation at 98°C (30 sec.), 30 cycles consisting of denaturation at 98°C (10 s), primer annealing at - 60°C (30 s), elongation at 72°C (60 s); and a final 72°C (10 min) pre-synthesis. PCR mix (25 μl) contained 4 μl HF Buffer (Thermo scientific), 0.5 μl 2 mM deoxyribonucleoside triphosphate (dNTP) mix, 10 pmol of each of the primers, 0.5 μl Phusion High-Fidelity DNA Polymerase (Thermo scientific) and 2 μl DNA as matrix. PCR was performed in a SimpliAmpTM thermocycler (Life Technologies Corporation). PCR products were separated by electrophoresis in 1% agarose gel using Tris-acetate-EDTA buffer and stained with ethidium bromide.

For termination amplification (Seq-PCR), primers with a concentration of 0.8 pm (picomole) and BigDye® Terminator kit v. 3.1 Cycle Sequencing Kit (Applied Biosystems, USA) were used. The reaction mixture was prepared according to the instructions provided by the manufacturer of this kit. BigDye®XTerminator™ Purification Kit (Thermo Fisher, USA) was used for purification

according to the instructions. The quality of reading of nucleotide sequences was determined using the Sequencing Analysis proframm. Homologous nucleotide sequences were searched using BLAST (Basic Local Alignment Search Tool) in the Gene Bank International Database of the National Centre for Biotechnology Information (htpp://www.ncbi.nlm.nih.gov).

***Results and discussion***

Monitoring surveys of walnut plantations to detect pathogens of walnut diseases were conducted in 2022-2023 in the farms of Turkestan region - LLP "Saryagash zher syyy", Almaty region - LLP "Integration - Turgen" and State Enterprise "Issyk State Dendrological Park".

The climate of Almaty region where the farms LLP "Integration - Turgen" and State Enterprise "Issyk State Dendrological Park" are located is sharply continental, winter is mild, summer is hot. The investigated site, located in the foothill zone of the south-eastern part of Saryagash district of Turkestan region on the territory of LLP "Saryagash zher syyy" is characterized by hot climate, low air humidity, abundance of sunlight, short winter.

During visual inspection of walnut samples, symptoms characteristic of lesions by bacterial and fungal pathogens were noted. In the State Enterprise "Issyk State Dendrological Park" in spring at relatively low temperatures and high air humidity on the leaves of walnut variety Liaohe of Chinese selection formed black spots, which appeared first on the edges and then gradually moved into the depth of the laminae (Figure 1a). Black spots were observed on branches, petioles, fruits and flowers, to which the pathogen moved from leaves through the vascular system. Then leaves twisted along the central vein in the form of a boat, turned dark, dried up, and in this form remained hanging on the tree for a long time (Figure 1 b, c). Cracks were formed on the bark of trunks at the base of skeletal branches and on small branches. Necrotic black spots were recorded on stems (Figure 1 d). With the onset of heat the disease development was suspended, the affected bark turned brown, peeled off, tissues were pushed in, forming a noticeable boundary separating healthy bark from diseased one. All these symptoms were characteristic of bacterial spots disease, caused by the bacterium *Pseudomonas syringae*. Small, dark spots on leaves caused by the pathogen *Pantoea agglomerans* were also observed on the variety Liaohe of Chinese selection (Figure 1 e).

In LLP "Integration - Turgen" on introduced walnut varieties Miroslava, Ovidiu, Germisara, Yarivski, Peshanski, Kogylnichanu, 185, Lipin 2, Liaohe, Fernor, Plin, Kishinevski, Chernovitski, Velnitsa, Xin Xin 2, Jin Long 2, Milotai 10, CITH - W2, Lara, Lateral lui Trifan, Brichanski, Codrene were recorded leaves with necrotic dark spots (Figure 1 f). Young shoots had brown and necrotic lesions. These symptoms were characteristic of walnut blight disease caused by the bacterium *Pantoea agglomerans*. Also, on introduced walnut varieties Liaohe, Peshanski, Velnitsa, Kohozev, Lateral lui Trifan, Brichanski, Kishinevski, Plin, Codrene, symptoms of bacterial spot disease caused by the bacterium *Pseudomonas oryzahabitans* were observed. Leaf spots were recorded predominantly along the margins of leaflets (Figure 1 c), occasionally between veins. Lesions were initially soft and rotten, then light brown, rounded or semicircular. Subsequently, neighboring foci merged, and symptoms of browning and wilting appeared on margins and whole leaves.

In Turkestan region, the first signs of infestation on local forms of Saryagash started from inflorescences and spread to shoots and branches. Walnut shoots turned brown, and brown, necrotic spots of different shapes were recorded on leaves (Figure 1k). All these symptoms were characteristic of walnut blight, caused by the bacterium *Pantoea agglomerans*. In June, when the weather became hotter, the disease became less active and the outbreak stopped. From this period until October there was dry weather, a clear moisture deficit with no rainfall and high temperatures. As a consequence, the degree of thermal damage to trees increased and leaves with desiccation and chlorosis were found.

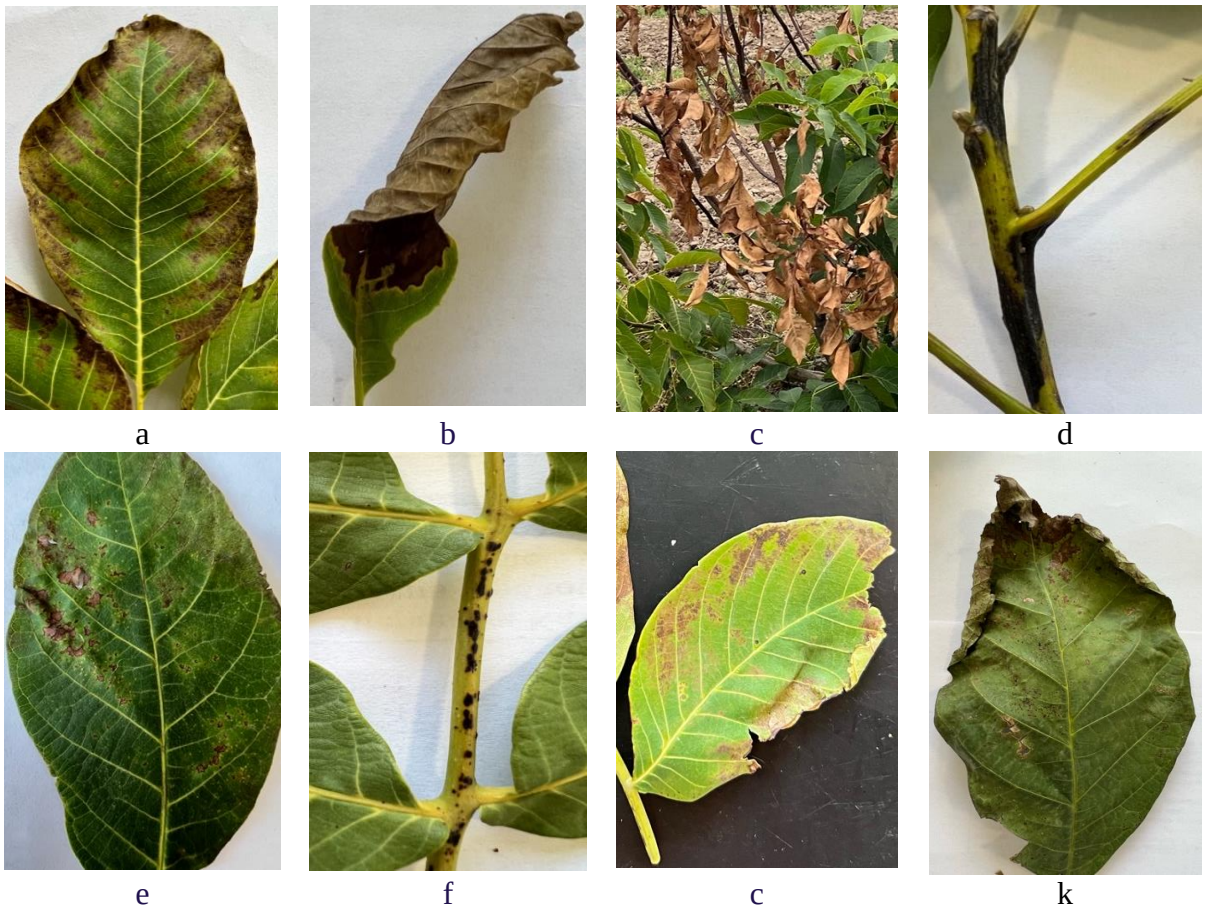
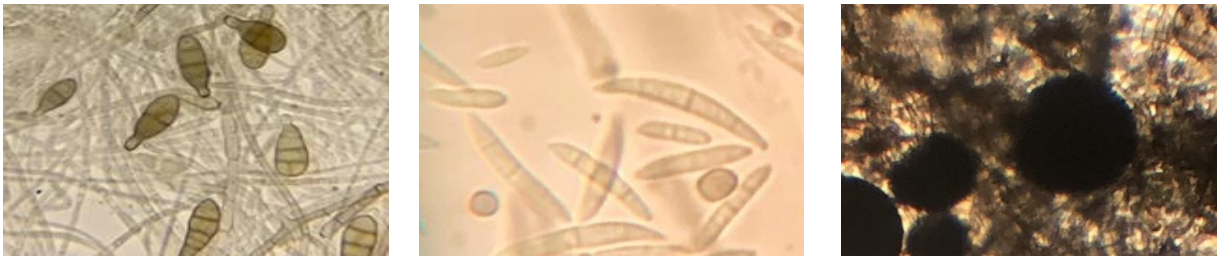


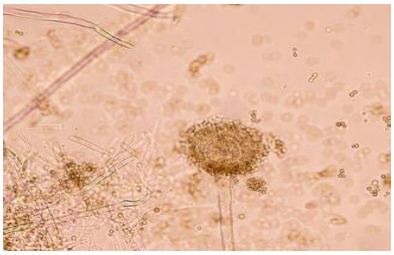
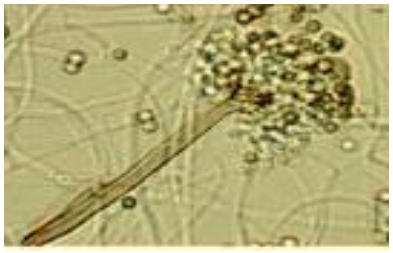
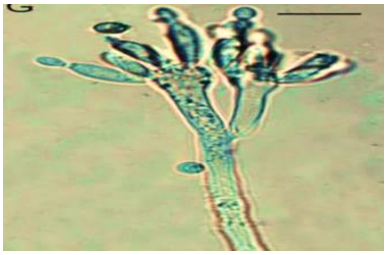

**Figure 1 -** Symptoms of manifestation of bacterial diseases on walnut in the southern zone of fruit growing in Kazakhstan (a, b, c, d - *Pseudomonas syringae*; e, f - *Pantoea agglomerans* from Almaty region; c - *Pseudomonas oryzahabitans*; k - *Pantoea agglomerans* from Turkestan region)

In order to identify walnut diseases by isolating the pathogen into pure cultures, microbiological analyses of leaf, stem, fruit and bark samples taken as a result of the survey were carried out in laboratory conditions. To identify pathogens, 99 samples from local forms and 81 samples from introduced walnut varieties with disease symptoms were taken.

At microscopy, pure cultures of fungi isolated from samples of leaves, stems and fruits of walnut from farms of Almaty region (LLP "Integration - Turgen" - introduced varieties Miroslava, Ovidiu, Germisara, Yarivski, Peshanski, Kogylnichanu, 185, Lipin 2, Liaohe, Fernor, Plin, Kishinevski, Chernovitski, Velnitsa, Xin Xin 2, Jin Long 2, Milotai 10, CITH - W2, Lara, Lateral lui Trifan, Brichanski, Codrene; The State State Enterprise "Issyk State Dendrological Park" - variety Liaohe), and Turkestan region (LLP "Saryagash zher syyy" - local forms Saryagashsky), were identified as *Alternaria Alternata, Fusarium solani,* and fungi *Aspergillus niger, Penicillium citrinum, Aspergillus flavus, Aspergillus calidoustus, Penicillium oxalicum, Alternaria angustiovoidea* as secondary infestation according to morphological features of mycelium and fungal sporulation (Figure 2).



*Alternaria alternata* *Fusarium solani* *Aspergillus niger*

*Aspergillus flavus* *Aspergillus calidoustus* *Penicillium oxalicum*

**Figure 2 -** Colonies of fungal microflora isolated from walnut samples

It was found, that the fungus of the genus *Alternaria* is the most frequent fungal pathogen on walnuts in the conditions of the southern zone of fruit growing in Kazakhstan (Table 1).

**Table 1 -** Fungal microflora detected on walnut samples taken from introduced varieties and local forms in farms of Almaty and Turkestan regions

| Fungi isolated from walnut samples | Stems | Leaves | Fruits | Number of isolates examined |
|---|---|---|---|---|
| *Alternaria alternata* | + | + | + | 30 |
| *Fusarium solani* | + | + | + | 20 |
| *Aspergillus niger* | + | + | | 9 |
| *Aspergillus flavus* | + | + | | 9 |
| *Aspergillus calidoustus* | | + | | 6 |
| *Penicillium oxalicum* | + | + | | 8 |
| *Penicillium citrinum* | | + | | 3 |
| *Alternaria angustiovoidea* | | + | | 5 |
| Total | | | | 90 |

In order to detect bacterial diseases of walnut by isolating the causative agent of the disease in pure cultures in laboratory conditions, bacteriological analyses were carried out on samples of stems, leaves and bark taken as a result of a survey of farms: Almaty region - LLP "Integration - Turgen" from introduced varieties Miroslava, Ovidiu, Germisara, Yarivski, Peshanski, Kogylnichanu, 185, Lipin 2, Liaohe, Fernor, Plin, Kishinevski, Chernovitski, Velnitsa, Xin Xin 2, Jin Long 2, Milotai 10, CITH - W2, Lara, Lateral lui Trifan, Brichanski, Codrene, from the State Enterprise "Issyk State Dendrological Park" from the variety Liaohe, from Turkestan region - LLP "Saryagash zher syyy" from local forms of Saryagashsky. Bacteria isolated from plant fragments with signs of bacterial blight (*Pantoea agglomerans*) on Nutrient agar had vague, slimy, shapeless, yellowish colonies (Figure 3a). Bacteria isolated from plant fragments with evidence of bacterial spot (*Pseudomonas syringae*) on Nutrient agar had smooth, flat, beige-coloured colonies (Figure 3b). Bacteria isolated from plant fragments with signs of bacterial spot (*Pseudomonas oryzahabitans*) on Nutrient agar had rounded shape, yellow colour, with raised shiny surface and smooth edge colonies (Figure 3 c).

*Pantoea agglomerans* a | *Pseudomonas syringae* b | *Pseudomonas oryzahabitans* c

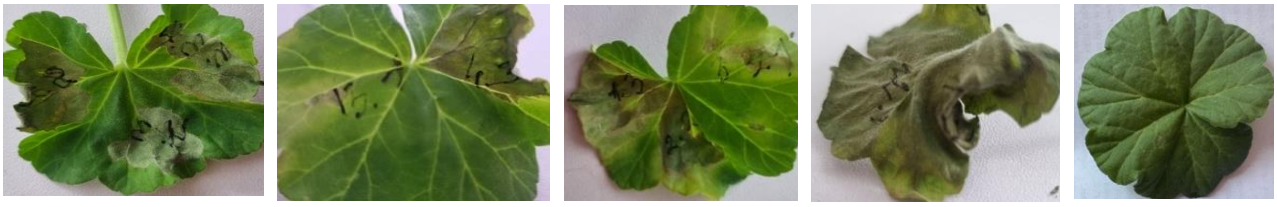
**Figure 3 -** Typical morphology of bacterial colonies isolated from walnut samples on Nutrient agar

In bacteriological analysis, *Pseudomonas syringae* was best isolated from bark taken at the border of wedge canker, on fruits, as well as from bent tips of young shoots from the variety Liaohe in the State Enterprise "Issyk State Dendrological Park". Isolates of the bacterias, *Pantoea agglomerans* and *Pseudomonas oryzahabitans* were isolated mainly from walnut leaves from farms of Almaty region - LLP "Integration - Turgen" from introduced varieties Miroslava, Ovidiu, Germisara, Yarivski, Peshanski, Kogylnichanu, 185, Lipin 2, Liaohe, Fernor, Plin, Kishinevski, Chernovitski, Velnitsa, Xin Xin 2, Jin Long 2, Milotai 10, CITH - W2, Lara, Lateral lui Trifan, Brichanski, Codrene and from Turkestan region - LLP "Saryagash zher syyy" from local forms Saryagashsky (Table 2).

**Table 2 -** Bacterial microflora detected on walnut samples

| Bacteria isolated from walnuts | Stems | Leaves | Fruits | Bark | Number of isolates examined |
|---|---|---|---|---|---|
| *Pantoea agglomerans* | + | + | + | | 29 |
| *Pseudomonas syringae* | + | + | + | + | 12 |
| *Pseudomonas oryzahabitans* | + | + | | | 9 |
| Total isolate | | | | | 50 |

To confirm the pathogenicity of bacteria *(Pantoea agglomerans, Pseudomonas syringae, Pseudomonas oryzahabitans),* we tested the infection-infiltration method of Clement's hypersensitivity reaction on the tested leaves of indoor geranium *(Pelargonium zonala L.).* As a result of testing the pathogenic properties of bacteria on the test object, tissue necrosis was obtained at the sites of inoculum introduction (Figure 4). This is a hypersensitivity reaction of the indicator plant to pathogenic bacterial species. Non-pathogenic bacteria do not cause such a reaction, even when high concentrations of inoculum are introduced. Thus, positive test results indicate that the bacteria are pathogenic.



a b c d e

**Figure 4** - Testing of the pathogenic properties of bacteria isolated in pure culture (a - *Pantoea agglomerans* from Almaty region, b - *Pantoea agglomerans* from Turkestan region, c - *Pseudomonas syringae,* d - *Pseudomonas oryzahabitans,* e - control) on geranium leaves *(Pelargonium zonala L.).*

Identification of fungal and bacterial diseases was carried out on the basis of PCR analysis, confirming the presence of pathogens in plants. The ITS (Internal Transcribed Spacer) site was used as a molecular genetic marker for fungi and 16S rRNA for bacteria. These markers are characterized by relatively high conservativity, good study, and a large number of nucleotide sequences in open databases.

For molecular genetic identification, DNA was isolated from 11 pure cultures of micromycetes and from 4 bacterial isolates isolated from diseased walnut plants. The DNA isolated from them was amplified with universal primers ITS1 / ITS4 and 704 F /1495 R. The amplification resulted in fragments of about 450-700 base pairs for the primer pairs, 700-800 base pairs for 704 F /1495 R, which corresponds to the expected size (Figure 5).

Positive amplicons of the ITS gene and 16S rRNA from the PCR product were purified with EXOSAP-IT PCR product purification reagent (Thermo Scientific) and sequenced with a sequencing kit, in both directions using the same Sanger universal primers on a 3500xL Genetic Analyzer (Applied Biosystems, Genetic Analyzer). The gene sequences of bacterial and fungal isolates were analyzed using BLASTN in the National Centre for Biotechnology Information (NCBI) GenBank database for identification purposes, and for fungal isolates, the BOLD system was additionally used.

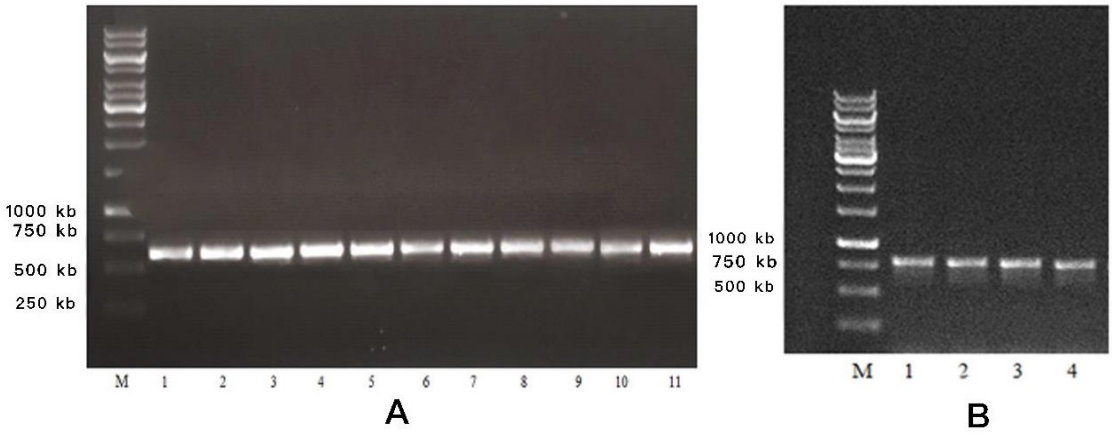



**Figure 5 -** PCR fragments obtained during DNA amplification of fungal isolates with primers A- ITS1/ITS4; and DNA of bacterial isolates with primers B- 704F/1495R.

M- Gene Ruler 1 kb DNA Ladder (Thermo Fisher, USA)

The DNA sequence homology of phytopathogenic fungi and bacteria is shown in Table 3.

**Table 3** - Homology of ITS gene and 16S RNA sequences of phytopathogenic fungi and bacteria

| Closest match to Genbank, sequence number | Coincidence with Genbank | |
|---|---|---|
| | Number of matching nucleotides | Match (%) |
| *Pantoea agglomerans* | 552/560 | 98,57 |
| *Pseudomonas syringae* | 736/748 | 98,40 |
| *Pseudomonas oryzihabitans* | 705/720 | 97,92 |
| *Alternaria alternata* | 500/500 | 100 |
| *Fusarium solani* | 479/480 | 99,79 |

| | | |
|---|---|---|
| *Aspergillus niger* | 519/519 | 100 |
| *Aspergillus flavus* | 450/450 | 100 |
| *Aspergillus calidoustus* | 494/496 | 99,60 |
| *Penicillium oxalicum* | 438/448 | 99,77 |
| *Penicillium citrinum* | 467/467 | 100 |
| *Alternaria angustiovoidea* | 499/500 | 99,80 |

As a result of PCR analysis, the bacterial pathogens were confirmed using primers 704F/1495R (Figure 5). The data obtained according to the described PCR methods confirmed the presence of *Pantoea agglomerans, Pseudomonas syringae, Pseudomonas oryzahabitans.*

As a result of PCR analysis, fungal pathogens were confirmed using primers ITS1/ITS4 (Figure 5). The data obtained according to the described PCR methods confirm the presence of certain fungal species such as *Alternaria alternata, Fusarium solani, Aspergillus niger, Aspergillus flavus, Aspergillus calidoustus, Penicillium citrinum, Penicillium oxalicum, Alternaria angustiovoidea.*

***Conclusions***

Taking into account that walnut is becoming increasingly important as a fruit crop in our country, this article is the beginning of a more comprehensive study of phytopathogenic fungi and bacteria causing harmful diseases of walnut in the southern zone of fruit growing in Kazakhstan. So far in Kazakhstan there is no data in this direction, so future research will be directed to work on determining the varietal resistance of walnut to them and finding ways to fight against pathogens.

As a result of survey of walnut plantations of the southern zone of horticulture of Kazakhstan (Turkestan, Almaty regions) in 2022-2023 among introduced varieties and local forms, the presence of symptoms characteristic of bacterial and fungal diseases was noted. As a result of microscopic studies, the following fungi were isolated: *Alternaria Alternata, Fusarium solani*, which were similar to fungal pathogens by morphological and cultural features, as well as fungi *Aspergillus niger, Penicillium citrinum, Aspergillus flavus, Aspergillus calidoustus, Penicillium oxalicum, Alternaria angustiovoidea*, as secondary infestation. The bacteria *Pantoea agglomerans, Pseudomonas syringae, Pseudomonas oryzahabitans,* causative agents of bacterial infection were isolated from the bacterial infection. As a result of testing the hypersensitivity reaction of the indicator plant to pathogenic bacterial species, tissue necrosis was obtained in the places of inoculum introduction, which confirms their pathogenicity.

On the basis of the studies conducted for the identification of the causative agent of fungal and bacterial diseases by PCR, it was found that DNA isolated from cultures of micromycetes and bacterial isolates was successfully amplified using universal primers ITS1/ITS4 and 704 F/1495 R. The fragments obtained corresponded to the expected sizes, confirming the efficiency of the primers used. Sequencing yielded gene sequences in both directions. The results of sequence homology obtained allowed accurate identification of microbial species. The homology of ITS and 16S rRNA gene sequences confirms the presence of phytopathogenic fungi *Alternaria alternata, Fusarium solani,* as well as phytopathogenic bacteria *Pantoea agglomerans, Pseudomonas syringae, Pseudomonas oryzihabitans* in the studied walnut samples.

The results demonstrate high identification accuracy, reflected in the high percentage of nucleotide matches for most of the species studied. Overall, this research provides important molecular genetic data necessary for a better understanding of the pathogenic flora affecting walnut, which may contribute to the development of effective methods to control these microorganisms and protect plants from diseases.

Since the studies are aimed at solving the issues of organization of production of competitive products, they will be in demand both from large producers of walnut fruit products and from peasant and private farms.

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**References**

1. Marketing research, market of nut crops for 2013-2017. OGAU "Innovation and consulting centre of agroindustrial complex". *Belgorod.* 2018. (in Russian).

2. Hasanov, Z.M.; Ibragimov, Z.A.; Sadygov, G.N.; Sardarova, D.I.; Alizadeh, R.A. Wild relatives of nut fruit crops in the Lesser Caucasus within Azerbaijan. *Contemporary horticulture.* 2016, *1,17,36-51*. (in Russian).

3. Kairova, G.N. Prospects of walnut production in Kazakhstan. Collection of materials of the international scientific and practical seminar "Providing the nut growing industry of Kazakhstan with high-quality planting material of promising forms and varieties". 2018, *6-9*. (in Russian).

4. Development of walnut growing in friendly countries. Available online: http://orehovod.com/. (in Russian)

5. Oleichenko, S.N. Bookmark walnut plantations in the South of Kazakhstan. Collection of materials of the international scientific and practical seminar "Providing the nut growing industry of Kazakhstan with high-quality planting material of promising forms varieties". 2018, *10-13.* (in Russian).

6. Konstantinova, M. Dangerous diseases of walnut. *Propozitsiya.* 2017, *5, 126-129.* (in Ukrainian).

7. Hassan, M.; Ahmad, K. Anthracnose disease of walnut - a review. *International Journal of Environment, Agriculture and Biotechnology.* 2017, *2, 5, 238-248.* DOI: 10.22161/ijeab/2.5.6

8. Cheng, A.; Liu, C.Y.; Tsai, H.Y.; Hsu, M.S.; Yang, C.J.; Huang, Y.T. et al. Bacteremia caused by *Pantoea agglomerans* at a medical center in Taiwan 2000 – 2010. *J. Microbiology Immunol. Infection*. 2013, *46, 187–194*. DOI: 10.1016/j.jmii.2012.05.005.

9. Dutkiewicz, J.; Mackiewicz, B.; Lemieszek, M.K.; Golec, M.; Milanowski, J. *Pantoea agglomerans:* a mysterious bacterium of evil and good. Part IV. Beneficial effects. *Ann. Agric. Environ. Med.* 2016, *23, 206–222*. DOI: 10.5604/12321966.1203879.

10. Yang, K.Q.; Qu, W.W.; Liu , X.; Liu , H.X.; Hou, L.Q. First report of *Pantoea agglomerans* causing brown apical necrosis of walnut in China. *Plant Dis.* 2011, *95(6):773.* DOI: 10.1094/PDIS-01-11-0060.

11. Belisario, A.; Maccaroni, M.; Corazza, L.; Balmas, V.; Valier, A. Occurrence and etiology of brown apical necrosis on Persian (English) walnut fruit. *Plant Disease.* 2002, *86: 599-602.*

12. Arquero, O.; Lovera, M.; Rodriguez, R.; Salguero, A.; Trapero, A. Characterization and development of necrotic lesions of walnut tree fruits in southern Spain. *Acta Horticulturae.* 2005, *705: 457-461.*

13. Moragrega, C.; Aleta, N.; Rovira, M.; Matias, J. Disease progress and microorganisms associated to brown apical necrosis of walnut. Files of the COST 873 WG1-4 Meeting: active research to combat bacterial diseases of stone fruits and nuts. *Athens*. 2008.

14. Huai, T.; Zhao, J.; Zhang, X.; He, H.; Zhu, X.; Ma, H.; Zhang, L.; Zhao, P.; Liu, X.; Si, D. First report of *Pseudomonas oryzihabitans* causing walnut leaf spot disease in China. *Plant Dis.* 2023. https://doi.org/10.1094/PDIS-08-23-1634-PDN.

15. Keshtkar, A.R.; Khodakaramian, G.; Rouhrazi, K. Isolation and characterization of *Pseudomonas syringae pv. syringae* which induce leaf spot on walnut. *Eur J Plant Pathol.* 2016, *146, 837–846.* DOI:10.1007/s10658-016-0962-2.

16. Programme and methodology of varietal studies of fruit, berry and nut crops. Under the general editorship of E. N. Sedov and T. P. Ogoltsova. *Orel: VNIISPK Publishing House.* 1999, *102-111.* (in Russian).

17. Chebanenko, S.I. Practicum on forest phytopathology: Textbook / S.I. Chebanenko, O.O. Beloshapkina. - Moscow: Izd. RGAU-MSKHA. 2012. *102.* (in Russian).

18. Chumakov, A.E.; Minkevich, I.I.; Vlasov, Y.I.; Gavrilova, E.A. Basic methods of phytopathological research. *Moscow: Kolos.* 1974, *190.* (in Russian).

19. Chumaevskaya, M.A.; Matveeva, E.V. Methodical instructions for isolation and identification of phytopathogenic bacteria. *Moscow.* 1986. (in Russian).

20. Bergey, D.H.; Harrison, F.C.; Breed, R.S.; Hammer, B.W.; Huntoon, F.M. Bergey's manual of determinative bacteriology. *Baltimore: Williams and Wilkins.* 1923.

21. Klement, Z.; Rudolph, K.; Sands, D.S. Methods in phytobacteriology. *Budapest: Akademia kiado* 1990, *568.*

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## ҚАЗАҚСТАННЫҢ ЖЕМІС ӨСІРУДІҢ ОҢТҮСТІК АЙМАҒЫНДАҒЫ ГРЕК ЖАҢҒАҒЫНЫҢ НЕГІЗГІ АУРУЛАРЫН АНЫҚТАУ

***Аңдатпа***

Қазақстан Республикасында жаңғақ дақылдарын өнеркәсіптік өсіруге қолайлы топырақ-климат жағдайлары, әсіресе оңтүстік өңірдің қолайлылығы ықпал етеді, бұл болашақта осы бағалы өсімдіктің жоғары өнімін алуға мүмкіндік береді. Жаңғақ дақылдарының өнімділігіне айтарлықтай зиян келтіретін аурулардан қорғау олардың өнімділігін арттыруға бағытталған негізгі шаралардың бірі болып табылады. Жаңғақ дақылдарын өсірудегі ең маңызды мәселе - оны бактериялық және саңырауқұлақ қоздырғыштарынан босату. Қазақстанда жаңғақ дақылдарының аурулары мен зиянкестерінің түрлік әртүрлілігін зерттеу бойынша мақсатты зерттеулер жүргізілген жоқ. Жүргізілген зерттеулердің жаңалығы - Қазақстанда алғаш рет микробиологиялық және молекулалық-генетикалық әдістер негізінде жеміс өсірудің оңтүстік аймағы жағдайында грек жаңғағының саңырауқұлақ және бактериялық аурулары анықталды.

Зерттеу нәтижелері оқшауланған ауру қоздырғыштардың көпшілігі саңырауқұлақтар, сондай-ақ жаңғақ ауруын тудыратын бактериялардың бірнеше түрі екенін көрсетті. Саңырауқұлақтардың 4 тұқымдары: *Alternaria, Fusarium* - саңырауқұлақ ауруларының қоздырғыштары; *Aspergillus, Penicillium* - қайталама популяция ретінде; сонымен қатар бактериялардың 2 тұқымдары: *Pantoea, Pseudomonas*, бактериялық аурулардың қоздырғыштары ретінде анықталды. Геномдық ДНҚ негізіндегі ПЦР арқылы саңырауқұлақтар мен бактериялық аурулардың қоздырғыштарын молекулалық сәйкестендіру зерттелген грек жаңғағының үлгілерінде фитопатогенді саңырауқұлақтар ретінде: *Alternaria alternata, Fusarium solani*, сонымен қатар фитопатогенді бактериялар: *Pantoea agglomerans, Pseudomonas syringae, Pseudomonas oryzahabitans* қоздырғыштары анықталғанын көрсетті. Жүргізілген зерттеу нәтижелері грек жаңғағының өнімділігін арттыру мақсатында оларды зиянды аурулардан қорғау жүйесін құруға мүмкіндік береді.

***Кілт сөздер:*** *грек жаңғағы, бактериялық аурулар, саңырауқұлақ аурулары, патогенділігін талдау, молекулалық сәйкестендіру, геномдық ДНК, ПЦР талдау*

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# ВЫЯВЛЕНИЕ ОСНОВНЫХ БОЛЕЗНЕЙ ГРЕЦКОГО ОРЕХА В ЮЖНОЙ ЗОНЕ ПЛОДОВОДСТВА КАЗАХСТАНА

***Аннотация***

Промышленному выращиванию орехоплодных культур в Республике Казахстан способствуют благоприятные почвенно-климатические условия, особенно в южном регионе, что позволяет в перспективе получать здесь высокие урожаи этого ценного растения. Значительный ущерб урожаю орехоплодных культур причиняют болезни, защита насаждений от которых является одним из основных мероприятий, направленных на повышение их продуктивности. Важнейшим вопросом в выращивании орехоплодных культур является освобождение его от бактериальных и грибных патогенов. В Казахстане целенаправленных исследований по изучению видового разнообразия болезней и вредителей орехоплодных культур не проводилось. Новизна проведенных исследований заключается в том, что впервые в Казахстане выявлены грибные и бактериальные болезни грецкого ореха в условиях южной зоны плодоводства на основе микробиологических и молекулярно-генетических методов.

Результаты исследований показали, что большинство выделенных патогенов представляют собой грибы, а также несколько видов бактерий, вызывающие болезни грецкого ореха. Всего выделено 4 рода грибов: *Alternaria, Fusarium* - возбудители грибных болезней*; Aspergillus, Penicillium* как вторичное заселение; также 2 рода бактерий: *Pantoea, Pseudomonas,* как возбудители бактериальных болезней. Молекулярная идентификация возбудителей грибных и бактериальных болезней методом ПЦР на основе геномной ДНК показала, что исследуемые образцы грецкого ореха были идентифицированы как фитопатогенные грибы: *Alternaria alternata, Fusarium solani*, а также фитопатогенные бактерии: *Pantoea agglomerans, Pseudomonas syringae, Pseudomonas oryzahabitans*. Проведенные результаты исследований позволят составить систему защиты грецкого ореха от вредоносных болезней с целью повышения продуктивности насаждений.

***Ключевые слова*****:** *грецкий орех, бактериальные болезни, грибные болезни, проверка на патогенность, молекулярная идентификация, геномная ДНК, ПЦР анализ*