

## ANNOTATION

**the dissertation work of Aitymbet Zhankeldy on the topic: «Mycoses of industrial orchards and apple forests and ways to reduce their harmfulness in the south-east of Kazakhstan», submitted for the degree of Doctor of Philosophy (PhD) on the educational program 8D08104 - Plant Protection and Quarantine**

**Relevance of the research topic.** Apple tree is the most widely cultivated fruit crop in commercial and private orcharding in southeastern Kazakhstan. Its popularity is driven by the high sensory quality of the fruit as well as its dietary and health-promoting properties. In addition to readily digestible sugars, apples contain salts of iron, sodium, manganese, potassium, phosphorus, and calcium, as well as citric, salicylic, boric, and other organic acids. In recent years, ensuring the domestic supply of locally produced fruit has become an important national objective. The most favorable bioclimatic conditions for growing apple, pear, apricot, and grape are found in the southern and southeastern regions of the republic—particularly in farms of the Almaty, Jetisu, Zhambyl, and Turkistan regions.

Within the overall structure of pome and stone-fruit plantings in Kazakhstan, apple orchards predominate, with total planted areas of about 36 thousand ha. In the Almaty region, the apple orchard area in 2019 amounted to 16,180.7 thousand ha, including 1.95 thousand ha of intensive orchards (13.5%). Fruit production reached 66.8 thousand tons with an average yield of 4.6 t/ha. At present, the area of intensive apple orchards in the Almaty region has reached 2,222 ha, and in 2022 it was planned to increase this figure to 2,600 ha. Intensive orchards cultivate varieties such as Fuji, Golden Delicious, Gala, Granny Smith, Starkrimson, Idared, and others. However, in commercial orchards and wild apple forests of southeastern Kazakhstan, yield growth dynamics remain low and do not meet quality requirements. Reduced yield and product quality are largely driven by fungal diseases, including powdery mildew, scab, moniliosis, and cytosporosis, which are among the most harmful.

Among the complex of fungal diseases, the most widespread and damaging across all apple-growing areas is apple scab (causal agent: *Venturia inaequalis* (Cooke) G. Winter). Yield losses due to scab are no less than 40%. Many researchers attribute the increasing harmfulness of scab in different regions to the long-term use of chemical plant protection products, which promotes natural selection of pesticide-resistant fungal strains, as well as to a general decline in the immune status of fruit plants caused by environmental pollution and changes in weather conditions. During epiphytotic years, this particularly dangerous and widespread disease may affect 65–97% of leaves and 80–100% of fruits on susceptible cultivars, causing fruit drop and, in some cases, complete crop loss.

Powdery mildew can reduce fruit yield in susceptible apple cultivars by 40–80%. Among apple fruit rots, moniliosis caused by *Monilinia fructigena* (Pers.) Honey is of major importance. In infected plants, newly formed ovaries may abscise. Chronic damage can negatively affect yield for many years.

Cytosporosis is a dangerous fungal infection capable of infecting fruit trees and causing substantial orchard losses. When trees are affected by this fungus, sap flow occurs mainly in the bark; if the bark is damaged, branches or sections of the trunk may dry out due to insufficient nutrient supply. Researchers have noted that in certain years this disease can be more harmful under Kazakhstan's conditions than all other apple diseases combined.

In recent years, global climate change and environmental pollution have strongly contributed to the increased distribution and severity of fungal diseases in industrial apple orchards and mountain forests. In addition, many older cultivars are becoming immunologically weaker against new fungal strains, while newer cultivars have not yet been fully evaluated for resistance to the most common fungal diseases.

Studies conducted abroad and in Kazakhstan have addressed many theoretical and applied aspects related to the biology of pathogens, disease development, spread of fungal diseases, and plant protection methods. However, a comprehensive investigation of apple diseases in industrial orchards and mountain forests of southeastern and southern Kazakhstan—including determination of pathogen species diversity, symptom characterization, evaluation of field resistance of cultivars, identification of resistance genes to the most harmful fungal diseases using molecular marker methods (PCR), and selection of the most resistant cultivars for commercial orcharding based on these data—has not previously been carried out. Therefore, research in this direction is currently timely and highly relevant.

**Purpose of the study.** The aim of the research was to study the prevalent fungal diseases of apple trees in commercial orchards and mountain forests of southeastern and southern Kazakhstan and to apply modern approaches to reduce their harmful effects.

**Research objectives:**

- To determine the species diversity of pathogenic fungi causing diseases of apple trees in commercial orchards and mountain forests of southeastern Kazakhstan and to characterize their biological properties;
- To provide a microbiological characterization and molecular identification of the pathogenic fungi *Venturia inaequalis* and *Podosphaera leucotricha*;
- To analyze the species composition of the mycobiota (fungal community) of Sievers apple (*Malus sieversii* (Ledeb.) M. Roem.) across the surveyed mountain forest areas of Kazakhstan;
- To investigate the prevalence and development of apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*) in southern and southeastern Kazakhstan and to identify apple varieties resistant to these diseases based on phytopathological assessment;
- To conduct molecular screening using DNA marker technologies to identify apple varieties carrying genes for resistance to fungal diseases;
- To determine the economic efficiency of cultivating apple varieties resistant to fungal diseases.

**Research methods.** To record the geographical coordinates of the sampling sites, a GPS device (Garmin) was used. Collection of herbarium material, drying procedures, and preparation of temporary mounts were carried out according to

generally accepted methodologies (Poliksenova V. D., Khramtsov A. K., and Piskun S. G.). For light microscopy of temporary mounts, a Polyvar photomicroscope equipped with Nomarski differential interference contrast (DIC) optics was used. The detected fungi were identified using appropriate identification keys.

A taxonomic checklist of fungi was compiled using the MycoBank database (Fungal Databases, Nomenclature & Species Banks). Fungal taxon names are given in accordance with the Index Fungorum database. Cultivar resistance to diseases was evaluated under natural field infection conditions in July 2022. The susceptible cultivars Golden Delicious and Idared were used as controls. Scab severity was assessed using the scale proposed by Chevalier et al. (1991). Twenty-five isolates of interest—because they resembled both scab (*V. inaequalis*) and *Alternaria* spp. (*Alternaria tenuis* Nees, *Alternaria alternata*)—were collected in July 2022 from commercial apple-growing areas in seven districts of southern and southeastern Kazakhstan, and single-spore isolations were obtained on water agar medium.

Germinated single spores were transferred to slants of potato dextrose agar (PDA), incubated at 20°C, and preserved for further use.

Molecular screening was performed based on PCR analysis using molecular markers. DNA was extracted from fresh leaves of different apple cultivars. The following markers were used to identify scab- and powdery mildew resistance genes Rvi2, Rvi4, Rvi5, Rvi6, Rvi8, Rvi9, Rvi11, Rvi14, Rvi15, Pl1, Pl-w, Pl-d, and Pl2: OPL19, CH05e03, Hi07h02, AL07-SCAR, AM19-SCAR, K08, HB09, CH02f06, AT20-450, EM M02, EM M01, OPN18, OPU02, dr70F/dr339R, and dr55F/dr336R (Patocchi A., Frei A., Frey J.E., Kellerhals M.). In this study, both the CTAB method and a silica-based sorbent method were used for DNA isolation. Extraction was carried out according to J.J. Doyle et al. (1987) and a modified protocol including additional sample purification. PCR and restriction products were analyzed by electrophoresis using 1.8% agarose gels in TAE buffer. Ethidium bromide-stained gels were visualized using a transilluminator (Bio-Rad Gel Doc XRT, USA). Subsequent sequencing of PCR products was performed at Denizli University (Turkey). The nucleotide sequences of the obtained fungal amplicons were analyzed using the BLAST tool in the open genetic database GenBank.

Data analysis was based on the results of molecular assays. Visualization of PCR products was performed by gel electrophoresis. The banding pattern of each amplified PCR product was scored as “+”, indicating the presence of resistance, or “–”, indicating the absence of the resistance gene. Genetic distance was estimated using PopGene software (version 1.32; Yeh et al.) by calculating the Dice coefficient. The resulting index was used to construct a dendrogram using the unweighted pair-group method with arithmetic mean (UPGMA). The dendrogram was generated in Molecular Evolutionary Genetics Analysis (MEGA) software (version 11; Tamura et al.).

#### **Main provisions submitted for defense:**

- The species of pathogenic fungi affecting apple trees in the mountain forests of southeastern Kazakhstan and in commercial orchards of southern and southeastern Kazakhstan were identified; their biological characteristics were determined, and the symptoms of fungal diseases of apple trees were characterized;

- The pathogenic fungi *Venturia inaequalis* and *Podosphaera leucotricha* were microbiologically characterized and molecularly identified;
- The species composition of the mycobiota of Sievers apple (*Malus sieversii*) was analyzed across the surveyed mountain forest areas of Kazakhstan;
- Based on phytopathological assessment and disease symptoms, apple varieties with the highest resistance to harmful fungal diseases were identified;
- Molecular screening of an extensive collection of apple varieties was conducted using DNA marker technologies, resulting in the identification of varieties carrying genes for resistance to fungal diseases that are recommended as valuable sources for breeding programs and for cultivation in commercial orchards;
- The economic efficiency of cultivating apple varieties resistant to fungal diseases was determined.

### **Description of the Main Research Results**

During the study, phytopathological monitoring was conducted in commercial apple orchards in southeastern Kazakhstan, which made it possible to determine the prevalence and severity of the major diseases affecting apple trees. The monitoring results revealed the patterns of occurrence of apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*), as well as their variability depending on cultivar characteristics and the environmental conditions of the study areas. A total of 64 fungal species were identified in the mycobiota of wild apple (*Malus sieversii*) populations, of which 21 species were characterized by high phytopathogenic potential. Mycological and molecular genetic studies of *Venturia inaequalis* and *Podosphaera leucotricha* were conducted, and their species identity was confirmed using PCR and DNA sequencing methods. The resistance of apple cultivars to scab and powdery mildew was evaluated, and the distribution patterns of the Rvi and Pl resistance genes were determined. The results obtained provide a scientific basis for assessing the phytosanitary status of commercial apple orchards, diagnosing disease-causing agents, and selecting disease-resistant cultivars.

Based on the results of scientific research and field evaluation, the most resistant apple cultivars to fungal diseases were identified for specific environmental conditions of the studied districts. The introduction of these cultivars into production will make it possible to reduce disease-related losses and may serve as one of the effective components of an integrated plant protection system.

The use of the recommended cultivars in breeding programs will contribute to increasing the resistance of future cultivars to harmful diseases. The introduction of resistant cultivars into production will help reduce the use of chemical plant protection products, facilitate the production of environmentally safe products, and increase yield, product quality, and the economic efficiency of apple production.

### **Justification of the novelty and importance of the obtained results.**

**Scientific novelty of the research.** For the first time, the species composition of the major fungal pathogens of apple trees was comprehensively determined in commercial apple orchards and natural mountain-forest ecosystems of southeastern Kazakhstan. The causal agents *Venturia inaequalis* and *Podosphaera leucotricha* were accurately identified using morphological, cultural, and molecular-genetic methods. The study of the mycobiota of Sievers apple (*Malus sieversii*) resulted in

the systematization of fungal species diversity and refinement of the mycobiota composition, comprising 64 fungal species, including 21 species with high phytopathogenic potential, for which ecological and biological characteristics were determined. Based on phytosanitary monitoring conducted during 2022–2024, the prevalence and development dynamics of apple scab and powdery mildew in commercial orchards of southern and southeastern Kazakhstan were comparatively assessed for the first time, and patterns of their regional and annual variation were established. Based on phytopathological assessment and molecular-genetic screening, apple varieties with high resistance to apple scab and powdery mildew were selected, and the distribution patterns of genotypes carrying Rvi and Pl resistance genes were determined. These genotypes can be used as valuable initial material for breeding programs. The economic efficiency of the studied apple varieties was comprehensively evaluated, demonstrating that apple varieties resistant to fungal diseases are characterized by higher productivity and profitability.

**Practical significance of the research.** Based on the obtained research results (field assessments), the apple cultivars most resistant to the specific ecological conditions of the studied districts and regions were identified, which makes it possible to recommend them as one of the measures to reduce disease harmfulness and enhance plant protection.

The use of the recommended cultivars in breeding will increase the resistance of future varieties to economically important diseases, while their introduction into commercial production will help limit the use of chemical plant protection products, obtain environmentally friendly produce, increase yield and fruit quality, and improve economic efficiency.

When planning the establishment of apple orchards, it is recommended— as one approach to reducing dependence on chemical control measures— to use approved and promising cultivars, taking into account those identified in this study as more resistant to apple scab and powdery mildew.

**Compliance with scientific development directions and state programs.**

The dissertation was carried out at the NAO “Kazakh National Agrarian Research University” within the framework of program AP13068068 “Grant funding for young scientists”, funded by the Ministry of Science and Higher Education of the Republic of Kazakhstan, on the topic “Molecular screening and selection of apple cultivars resistant to fungal diseases using DNA marker technologies”.

**Description of the doctoral candidate’s contribution to publication preparation.** During the implementation of the research goals and objectives, the author directly participated in field and laboratory experiments as well as in the collection of scientific data. The accuracy and reliability of the obtained results are high, since the data were processed using methods of mathematical statistics. The author completed the dissertation and made an active contribution to the preparation and publication of scientific papers.

According to the results of the scientific research, a total of 7 scientific publications have been published, including 2 articles in journals indexed in the Scopus database and ranked in the Q1 and Q2 quartiles; 2 articles in scientific

journals recommended by the Committee for Quality Assurance in the Field of Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan; and 3 articles published in the proceedings of international scientific and practical conferences. The author's h-index is 2.

**Structure and volume of the dissertation.** The total length of the dissertation is 140 pages. The dissertation consists of an introduction, a main part comprising 4 sections, a conclusion, recommendations for production, and an appendix. The dissertation includes 30 tables, 34 figures, and 1 appendix. The list of references comprises 289 works by domestic and foreign researchers.