ANNOTATION

of the dissertation work of Elmira Ismagulova Sovetovna
on the topic: "Phytopathological assessment of introduced walnut varieties and the development of protective measures against major diseases for the southern fruit growing zone of Kazakhstan"
submitted for the degree of Doctor of Philosophy (PhD)
Educational Program 8D08104 – «Plant Protection and Quarantine»

Relevance of the research topic

One of the key factors limiting the development of walnut cultivation is the high susceptibility of walnut plantations to fungal and bacterial diseases. Ongoing climate change, expansion of walnut plantation areas, and reduction in the genetic diversity of cultivated forms contribute to increased activity of phytopathogens and, consequently, to significant economic losses in the industry.

This issue is particularly acute in the Republic of Kazakhstan, where currently no pesticides are officially registered for walnut crop protection. According to the "List of Pesticides Permitted for Production (Formulation), Import, Storage, Transportation, Sale and Use in the Territory of the Republic of Kazakhstan for 2022–2031", plant protection products for walnut cultivation have not been registered. As a result, phytopathogenic organisms spread virtually unchecked, causing considerable damage to both commercial plantations and private farms.

To date, no systematic scientific studies have been conducted in the republic aimed at a comprehensive investigation of the phytopathological status of walnut plantations, identification of the major pathogens, and evaluation of resistance of introduced varieties and genotypes. The lack of scientific data significantly complicates the development of effective protection measures and constrains the development of walnut cultivation in Kazakhstan.

Thus, the current phytosanitary situation, characterized by the active spread of fungal and bacterial diseases, the absence of registered plant protection products, and insufficient scientific knowledge of the problem, determines the high scientific and practical relevance of this research. A comprehensive phytopathological analysis will enable identification of major disease agents, evaluation of varietal resistance, and development of scientifically substantiated disease management recommendations, contributing to sustainable industry development and reduction of economic losses.

Purpose of the Research

The aim of this study is to identify and comprehensively investigate the causative agents of major walnut diseases using microbiological and molecular genetic methods; to assess the genetic potential of resistance in walnut varieties and genotypes against economically significant pathogens; and to develop scientifically grounded recommendations for disease management aimed at improving breeding processes and increasing the productivity of walnut plantations.

Research Objectives

- To monitor walnut plantations in the southern fruit-growing zone of Kazakhstan to identify fungal and bacterial diseases;

- To study the prevalence, intensity, and dynamics of major walnut diseases under regional conditions;

- To isolate and microbiologically identify pure cultures of fungal and bacterial pathogens;

- To identify pathogens using molecular genetic markers;

- To conduct molecular genetic assessment of genetic diversity and population structure of commercially valuable introduced varieties, local forms, and wild walnut populations in Kazakhstan using SNP genotyping with the Axiom™ *J. regia* 700K array;

- To evaluate the resistance of promising walnut varieties and genotypes to key phytopathogens;

- To study the effectiveness of modern fungicides and antibacterial preparations against major walnut diseases;

- To develop scientifically substantiated recommendations for walnut protection from fungal and bacterial diseases considering agroecological conditions of southern Kazakhstan.

Research Methods

The study employed a complex of field, laboratory, microbiological, molecular genetic, and statistical methods. The phytopathological status of walnut plantations was assessed through field surveys conducted during 2022–2024 using visual diagnostics and disease severity scales (DSI).

Isolation and identification of fungal and bacterial pathogens were carried out using classical microbiological methods and molecular genetic analysis. Fungal isolates were identified using ITS markers, whereas bacterial isolates were identified based on the 16S rRNA gene region, followed by sequencing and comparison with the GenBank and BOLD databases.

The pathogenicity of isolates was evaluated on walnut leaves, fruits, shoots, and seedlings in accordance with Koch's postulates. Genetic diversity and population structure of varieties, local forms, and wild populations were analyzed using SNP genotyping. Statistical analysis included ANOVA, PCA, ADMIXTURE, AMOVA, and calculation of FST coefficients.

Main Provisions Submitted for Defense

- A complex of major fungal and bacterial pathogens of walnut has been identified in the southern fruit-growing zone of Kazakhstan, with *Pantoea agglomerans* and *Alternaria alternata* being dominant;

- The phytosanitary status of plantations is determined by the interaction of climatic factors and mixed (co-)infections enhancing disease severity;

- For the first time in Kazakhstan, pathogenicity of several bacterial and fungal isolates was confirmed using molecular genetic identification and experimental inoculation;

- Wild and local walnut populations of Kazakhstan form genetically distinct clusters and represent an important source of stress- and disease-resistance genes;

- A scientifically grounded approach to evaluating walnut resistance and selecting protection measures for southern Kazakhstan conditions has been developed.

Main Research Results

- Monitoring revealed a complex of fungal and bacterial diseases affecting leaves, shoots, bark, and fruits. Dominance of bacterial blight associated with *Pantoea agglomerans* and widespread brown spot caused by *Alternaria alternata* were established;

- Pure cultures of major pathogens were isolated and characterized, and their pathogenicity confirmed experimentally. Molecular identification was performed using ITS and 16S rRNA markers;

- For the first time, SNP genotyping of wild, local, and introduced forms of Juglans regia in Kazakhstan was conducted, revealing the uniqueness of national genetic resources and their intermediate position between eastern and western populations;

- Different levels of resistance of introduced and local varieties to bacterial and brown spot diseases were determined, with most varieties classified as resistant or moderately resistant;

- Laboratory evaluation of antibacterial preparations identified the most effective agents against major bacterial pathogens;

- Scientifically grounded recommendations for walnut protection in southern Kazakhstan were developed.

Scientific Novelty and Significance

- For the first time in Kazakhstan, a comprehensive study of the walnut phytopathogenic complex using microbiological and molecular genetic methods was conducted;

- The species composition of major fungal and bacterial pathogens in walnut agroecosystems was identified and characterized;

- Diagnostic profiles of phytopathogens based on PCR identification were developed;

- Resistance of local and introduced walnut varieties to key phytopathogens was evaluated;

- Genetic diversity and population structure of walnut in southern Kazakhstan were determined for the first time using SNP genotyping;

- Scientifically grounded protection recommendations were developed.

Compliance with State Programs

The dissertation was carried out at the Kazakh National Agrarian Research University within Budget Program 102 “Grant Financing of Scientific Research” under the sub-priority “Innovative biological research to increase productivity and resistance of plant varieties and animal breeds in agriculture.”

The study was conducted under Grant AP19677936 “Study of major walnut diseases and molecular genetic basis of resistance of promising varieties to economically significant pathogens.”

Personal Contribution of the Doctoral Candidate to Each Article

The doctoral candidate personally conducted field phytopathological surveys of walnut plantations, collected and performed laboratory analysis of samples, isolated pure cultures of pathogens, and carried out microbiological and molecular genetic identification of the causative agents. Experiments were performed to evaluate the effectiveness of fungicides and antibacterial preparations, including field trials and analysis of their biological efficacy. The author prepared scientific publications and presented the research results at scientific conferences.

1. **First evaluation of genetic diversity and population structure of wild and cultivated Juglans regia in Kazakhstan.** Collected samples of wild and cultivated forms of *Juglans regia* from different regions of Kazakhstan. Compared the genetic diversity of wild and cultivated forms.

2. Identification, characterization, and pathogenicity of fungal and bacterial pathogens of walnut (Juglans regia L.) in Kazakhstan. Collected walnut (*Juglans regia L.*) samples showing disease symptoms in various regions of Kazakhstan. Isolated fungal and bacterial pathogens from infected tissues. Characterized the biological and cultural features of the identified strains. Assessed the pathogenicity of isolates through artificial inoculation and symptom analysis. Identified the most aggressive walnut disease pathogens in Kazakhstan.

3. **Statistical analysis of the effectiveness of antibacterial preparations against bacterial diseases of walnut using ANOVA.** Developed an experimental design to evaluate the effectiveness of antibacterial preparations against walnut bacterial diseases. Conducted field or laboratory trials on infected plants. Collected quantitative data (disease severity, disease index, necrosis area, etc.). Performed statistical analysis using analysis of variance (ANOVA). Evaluated the significance of differences among treatments and identified the most effective control agents against bacterial diseases.

4. **Resistance of walnut varieties to walnut blight caused by Pantoea agglomerans in the southern horticultural zone of Kazakhstan.** Evaluated the resistance of walnut varieties to walnut blight (*Pantoea agglomerans*). Isolated and confirmed the pathogen. Organized artificial inoculation of plants (or monitored natural infection background). Assessed the level of infection using a resistance scale/disease index. Compared the response of different varieties under the conditions of southern Kazakhstan. Identified resistant and susceptible genotypes and formulated recommendations for breeding and establishment of commercial orchards.

5. **Efficacy of a range of fungicides against fungal diseases of walnut in the southern fruit-growing zone of Kazakhstan.** Conducted monitoring of fungal diseases of walnut in the southern fruit-growing zone of Kazakhstan. Isolated and identified major phytopathogenic fungi (including *Alternaria*, *Fusarium*). Selected and tested fungicides with different modes of action. Performed field and/or laboratory trials to evaluate disease development and calculated biological efficacy (%). Analyzed statistical significance of treatment differences and developed practical recommendations for orchard protection.

6. **Pathogenicity of the fungus Alternaria alternata on walnut (Juglans regia) in the southern fruit-growing zone of Kazakhstan.** Collected walnut (*Juglans regia L.*) leaf samples with symptoms of brown spot in southern Kazakhstan. Isolated *Alternaria alternata* from infected tissues. Performed morphological identification of isolates and confirmed species identity using PCR (molecular identification). Conducted artificial inoculation under controlled conditions. Evaluated pathogenicity (chlorosis, necrosis, brown spot development). Analyzed aggressiveness of the pathogen in different climatic zones (Almaty and Turkestan regions). Confirmed the threat of disease spread and its impact on yield.

7. **Efficacy of a number of fungicides against the walnut pathogen Alternaria alternata in the southern zone of fruit growing in Kazakhstan.** Isolated and identified *Alternaria alternata* from infected walnut plants. Selected several fungicides for testing. Conducted laboratory (in vitro) and field trials to evaluate their effectiveness. Assessed inhibition of mycelial growth and disease symptom development. Calculated percentage inhibition and biological efficacy. Identified the most effective fungicides for southern Kazakhstan and formulated practical protection recommendations.

8. Identification of the main diseases of walnut in the southern fruit-growing zone of Kazakhstan. Conducted phytopathological monitoring of walnut plantations in southern Kazakhstan. Identified the main fungal and bacterial diseases of walnut (*Juglans regia L.*). Performed visual diagnostics and sampling of infected material. Isolated disease pathogens and conducted morphological and, when necessary, molecular identification. Assessed disease prevalence and severity and systematized data on dominant regional diseases.

The study was conducted at

In Kazakh National Agrarian Research University, at the Kazakh-Japanese Innovation Center of the Kazakh National Agrarian Research University, at the Kazakh Research Institute of Plant Protection and Quarantine named after Zh. Zhiembayev, and at the Institute of Plant Biology and Biotechnology, Almaty, Kazakhstan.

In addition, two implementation certificates were obtained. The first was issued by Rural Farm “Manshuk”, where recommendations on the use of the most effective fungicides were adopted. The second was issued by the LLP “Fazenda UM”, where resistant walnut forms were planted on an area of 2 ha, and recommendations for walnut protection were also implemented.

Volume and Structure of the Dissertation

The dissertation comprises 127 pages and consists of an introduction, three chapters, a conclusion, practical recommendations, and appendices**.** The work contains 24 tables and 29 figures**.** The reference list includes 156 sources, of which 149 are foreign publications**.**