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## CONTENTS

### **Analysis of the Correlation Between Various Biological and Economic Indicators of Mulberry Silkworms (*Bombyx mori* L.) Introduced from Uzbekistan**

*Gudurat M. Bakirov, Ramiz G. Bakirli, Ayaz M. Mammadov, Dursun M. Adigozalova, and Safarli Kh. Khuzhamatov* 5

### **Bioremediation Potential of Indigenous Earthworms in Radionuclide-Contaminated Soils of the Absheron Peninsula**

*Aygun S. Suleymanova and Constantin Daniel Negut* 13

### **The Role of Chemistry in the Development of Agriculture**

*Nizami A. Akbarov and Kamala O. Iskandarova* 19

### **Molecular Screening of *Ustilago maydis* Fungus in Maize Genotypes**

*Surayya T. Ismayilova and Saltanat A. Aghayeva* 25

### **Investigation of the Genetic Basis of Wheat Resistance to Salt Stress**

*Maryam E. Eyyubova* 32











### **Initial Diagnosis of Barley Rust Disease Based on Visual and Morphological Signs**

*Saida N. Burjaliyeva* 38

<https://doi.org/10.54414/AHNE2687>

Research Article

# Analysis of the Correlation Between Various Biological and Economic Indicators of Mulberry Silkworms (*Bombyx mori* L.) Introduced from Uzbekistan

Gudurat M. Bakirov<sup>1</sup>  , Ramiz G. Bakirli<sup>1</sup>  , Ayaz M. Mammadov<sup>2</sup>  , Dursun M. Adigozalova<sup>3</sup>  , and Safarli Kh. Khuzhamatov<sup>4</sup>  

<sup>1</sup>Sheki Regional Scientific Center of ANAS, L. Abdullayev Street 24, AZ5500 Sheki, Azerbaijan

<sup>2</sup>Department of Natural Sciences, School of Advanced Technologies and Innovation Engineering, Western Caspian University, 17 A, Ahmad Rajabli Street, III Parallel, AZ1072 Baku, Azerbaijan

<sup>3</sup>Azerbaijan State Agricultural University, Ataturk Avenue 450, AZ2000 Ganja, Azerbaijan

<sup>4</sup>Scientific Research Institute of Sericulture. 1 Ipakchi Street, Shaykhantahur District, Tashkent 100024 Uzbekistan

## Abstract

In this study, eight mulberry silkworm (*Bombyx mori* L.) breeds imported from Uzbekistan were evaluated under laboratory conditions at the Sheki Regional Scientific Center of ANAS based on five key biological and productivity indicators: survival rate, viability, cocoon weight, feeding period, and silkiness. The results revealed significant differences among the breeds, with the highest productivity observed in the Marvarid and Markhamat breeds. Correlation analysis demonstrated positive and statistically significant relationships between survival rate and viability ( $r = 0.788$ ;  $p < 0.05$ ), cocoon weight ( $r = 0.824$ ;  $p < 0.05$ ), and silkiness ( $r = 0.941$ ;  $p < 0.01$ ). In contrast, negative correlations were identified between feeding period and survival rate ( $r = -0.775$ ;  $p < 0.05$ ), as well as silkiness ( $r = -0.881$ ;  $p < 0.01$ ). Overall, the survival rate showed a strong positive association with cocoon weight and silkiness, and a negative association with feeding period. These findings indicate that cocoon weight and silkiness are key productivity indicators, whereas a prolonged feeding period adversely affects productivity. Therefore, the Marvarid and Markhamat breeds are recommended as priority candidates for selection and breeding programs.

**Keywords:** mulberry silkworm, breed, hybrid, wet cocoon, silk, correlation

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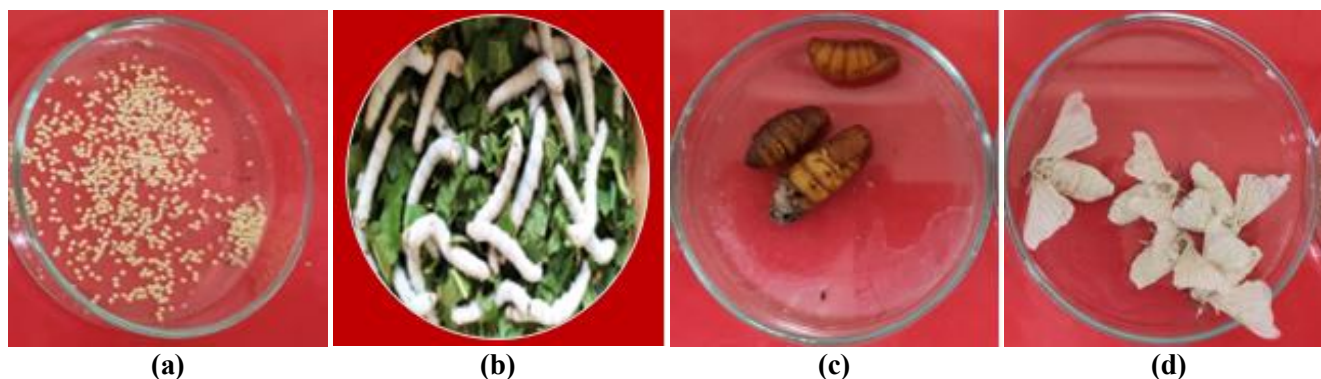
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## 1. Introduction

In the history of the development of sericulture in Azerbaijan, the northwestern region, its craftsmen, and the local population have had a unique place and role. The sericulture industry plays an important role in improving socio-economic conditions. The location of the sericulture industry on the “Great Silk Road” has led to the formation and development of this sector in Azerbaijan, especially in the northwestern region, and has given impetus to the expansion of trade and economic relations with other countries. Breeding science plays a major role in the development of sericulture and the achievement of new achievements. As a result of the application of scientific methodological selection, it is possible to create new breeds and lines.

Determining the biological indicators of introduced breeds under local conditions is essential for breeding programs. Because the viability and productivity of hybrids created between breeds taken from different geographical environments are high.

The life cycle of the silkworm goes through four developmental stages: egg, larva, pupa (chrysalis), and butterfly (adult) (Figure 1).



**Figure 1.** The life cycle of the silkworm: (a) egg, (b) larva, (c) pupa (chrysalis), (d) butterfly (adult).

The silkworm is considered an important source of raw material for the textile industry, and it is grown only in countries with favorable climatic conditions. The area where the production areas are located for the preparation of silk products using raw silk produced from the silkworm should also be selected according to the climatic conditions [1]. Azerbaijan is a country with such favorable climatic conditions, and the North-Western region is a region with the most favorable climatic conditions for the production of silk and silk products from the silkworm. This valuable wealth of the people, cultivated with hard work in the republic, should be used in such a way that the needs of the population are adequately met, and the population of the region is provided with employment. The former glory of Azerbaijani silk should be restored, and it should take its rightful place in the world market.

The "Azad" breed created by Professor R. A. Huseynov played a significant role in the republic's sericulture and had a significant positive impact on the economy of Azerbaijan with its good adaptation to local conditions, endurance, high productivity, and technological quality of the silk, etc., positive features. In the past, the Azad, Sheki-1, and Sheki-2 breeds in their pure form constituted 40-50% of the republic's total forage. However, considering the superiority of hybrids over pure breeds and the fact that only hybrids are currently used in forage farms, to further increase the productivity of these breeds, the most important issue of the day was to identify worthy components for the breeds existing in the republic and ensure their use in hybridization [2].

During family breeding, which is widely used in the selection and breeding of mulberry silkworms, 3 types of phenotypic variability of quantitative traits are observed in the selection material (in populations, lines, or breeds): intra-family, inter-family, and general variability. However, these have not been studied by anyone so far [3]. B. H. Abbasov's studies showed that among the 3 phenotypic variability parameters of 5 biological and 8 technological quantitative traits of the mulberry silkworm, the general variability parameters have the largest values, and the interfamilial variability parameters have the smallest values [1], [2]. He determined that the heritability coefficient of the mass of the live cocoon and silkiness decreases in the summer and autumn seasons compared to the spring season. Based on three-year experiments conducted at the Ganja breeding sericulture station on the elite material of the Azad and Ganja-1 breeds, which are regionalized in our republic, the heritability coefficient of the mass of the live cocoon and silkiness, as well as the mass of the cocoon in the cocoon, was determined by 3 methods [3].

A positive correlation was found between temperature and wet cocoon shell weight ( $r=0.383$ ), yield per box ( $r=0.357$ ), cocoon undrawn portion ( $r=0.345$ ), dry cocoon weight ( $r=-0.217$ ), cocoon width ( $r=-0.238$ ), silk richness ( $r=-0.255$ ) and silk yield ( $r=-0.256$ ); and a positive correlation was found between humidity and cocoon width ( $r=0.213$ ), fiber thickness ( $r=0.271$ ) and drawable yarn weight ( $r=0.201$ ) ( $P>0.05$ ). A negative correlation was found between humidity and silk richness ( $r=-0.287$ ) ( $P>0.05$ ). A negative correlation was observed between light and cocoon width ( $r=-0.385$ ), filament negation ( $r=-0.317$ ) and removable part weight ( $r=-0.410$ ), wet cocoon weight without chrysalis ( $r=-0.237$ ), cocoon length ( $r=-0.246$ ), cocoon width ( $r=-0.385$ ) and filament negation ( $r=-0.317$ ); a negative correlation was observed between carbon dioxide and silk richness ( $r=-0.560$ ) and the percentage of cocoons with silk ( $r=-0.350$ ) ( $P>0.05$ ). A negative correlation was found between carbon dioxide

and silk richness ( $p < 0.05$ ). A positive correlation was found between carbon dioxide and the weight of the non-removable part of the cocoon ( $r = 0.289$ ) ( $P > 0.05$ ) [4].

Sharma et al. (2020) reported that temperature and humidity showed a negative correlation with cocoon yield, with values of -0.72 and 0.52, respectively [5].

In the study conducted by Safarali Khudjamatov et al., the correlation between the duration of the larval period and egg-laying traits of 6 breeds and 4 silkworm breeding lines was studied. Breeds and lines were divided into grades according to the speed of development, and a comparative assessment of the indicators in the grades was carried out. Based on the analysis of the results obtained, a negative correlation was established between the speed of the larval period and reproductive characteristics [6].

In studies conducted by Hemmatabadi et al., the interaction between the genetic potential of silkworm species and the environment determines the silkworm's production capacity [7].

Elite and industrial seeds imported from abroad are superior to local varieties and hybrids in terms of quality, hatchability, biological and technological indicators, and most importantly, the short larval period of caterpillars. Therefore, reducing the larval period and creating highly productive varieties and hybrids are urgent tasks in sericulture. Scientists believe that an increase in productivity indicators is observed in populations with a long life cycle. However, foreign experience shows that it is possible to create rapidly growing and developing, as well as highly productive breeds and hybrids, using special selection methods. Based on this, at the current stage of development of the silkworm industry, the creation and application of breeds with a short life cycle and rapid cocoon collection in production is a priority task for genetic and selection researchers [8].

According to the selection results of the Guzal and Marvarid silkworm breeds, cocoons are divided into large, medium, and small cocoons in terms of size. The technological indicators of medium-sized cocoons are considered the best technological indicators. The existence of an inverse correlation between cocoon size and selection traits has been proven [9]. Pearson correlation was used to test the correlation between larval and cocoon parameters. A strong, positive, and highly significant correlation was observed between larval weight and length ( $R = 0.82$ ) and cocoon and pupa weight ( $R = 0.981$ ), and a moderate, highly significant correlation was observed between larval and silk gland weight ( $R = 0.601$ ), silk gland and shell weight ( $R = 0.622$ ), cocoon and shell weight ( $R = 0.694$ ), and shell and pupa weight ( $R = 0.552$ ) [10].

In a study conducted by Ridvan Yakisan and Ayhan Yilmaz, the relationship between some environmental measurements and cocoon productivity and quality characteristics was investigated. A positive correlation was found between temperature and wet cocoon shell weight ( $r = 0.383$ ), productivity per box ( $r = 0.357$ ), non-removable part of cocoon ( $r = 0.345$ ), dry cocoon weight ( $r = -0.217$ ), cocoon width ( $r = -0.238$ ), silk richness ( $r = -0.255$ ) and reza ( $r = -0.256$ ); and a positive correlation was found between humidity and cocoon width ( $r = 0.213$ ), filament negation ( $r = 0.271$ ) and removable yarn weight ( $r = 0.201$ ) ( $P > 0.05$ ). A negative correlation was found between humidity and silk richness ( $r = -0.287$ ) ( $P > 0.05$ ). There was a negative correlation between light and cocoon width ( $r = -0.385$ ), filament denier ( $r = -0.317$ ) and retractable part weight ( $r = -0.410$ ), wet cocoon weight without chrysalis ( $r = -0.237$ ), cocoon length ( $r = -0.246$ ), cocoon width ( $r = -0.385$ ), filament denier ( $r = -0.317$ ); carbon dioxide ratio was found to be negatively correlated with silk richness ( $r = -0.560$ ) and percentage of cocoon with fiber ( $r = -0.350$ ) ( $P > 0.05$ ). A negative correlation was found between carbon dioxide and silk richness ( $p < 0.05$ ). A positive correlation was found between carbon dioxide and non-retractable part weight of cocoon ( $r = 0.289$ ) ( $P > 0.05$ ). Sharma et al. They reported in their study (2020) that temperature and humidity were negatively correlated with cocoon productivity, with values of -0.72 and 0.52, respectively [4].

In recent decades, *Bombyx mori* L. has been successfully used as an alternative invertebrate model organism in various scientific fields. These applications include human disease modeling, environmental monitoring, epigenetic studies, as well as microbial drug testing and discovery [11].

## 2. Materials and Methods

The research work was carried out at the “Mulberry Silkworm Breeding Department” of the Sheki Regional Scientific Center of ANAS. The research was conducted in different seasons. Seeds of different breeds were incubated in both spring and summer. Different breeds were fed by families, and their biological indicators were determined.

The influence of weather and climate conditions on silkworms, especially on the efficiency of mass breeding, is one of the main factors. It shows the importance of a comprehensive study of the problem of genotype interaction and the need for targeted research. The following breeds were imported from the Institute of Sericulture of Uzbekistan in 2023.

1. *Gulshan*
2. *Orzu*
3. *Yulduz*
4. *Asaka*
5. *Guzal*
6. *Markhamat*
7. *Marvarid*
8. *Nafis*

The above breeds were fed in the spring and summer of 2023. The scientific research work was carried out in the “Selection of Mulberry Silkworm” department of the Sheki Regional Scientific Center. The following work was carried out in accordance with the methodology for the years.

In order to create a highly productive mulberry silkworm breed, the biological indicators of breeds imported from Uzbekistan were determined, and parental forms were determined to create new productive hybrids. The above breeds were fed by families, worms were counted after the third age, and fed in four repetitions with 200 worms each. The biological indicators of mulberry silkworms were studied.

In conducting the work, the Gulshan, Orzu, Yulduz, Asaka, Guzal, Markhamat, Marvari and Nafis mulberry silkworm breeds introduced in the selection work were used as the research object.

## 3. Statistical Analysis

The aim of the study is to determine the correlation between viability, cocoon weight, and silkiness.

The correlation coefficient between the biological and economic characteristics of the silkworm is calculated using the following formula:

$$r = \frac{\sum(X - \bar{X})(Y - \bar{Y})}{\sqrt{\sum(X - \bar{X})^2 \cdot \sum(Y - \bar{Y})^2}}$$

Here,  $r$  is the correlation coefficient,  $X$  and  $Y$  are the variables, and  $\bar{X}$  and  $\bar{Y}$  are the mean values of the variables. The correlation coefficient was also calculated using SPSS, and the “ $r$ ” table was used to determine the statistical significance of the results.

The degree of freedom is calculated by the formula:

$$df = n - 2$$

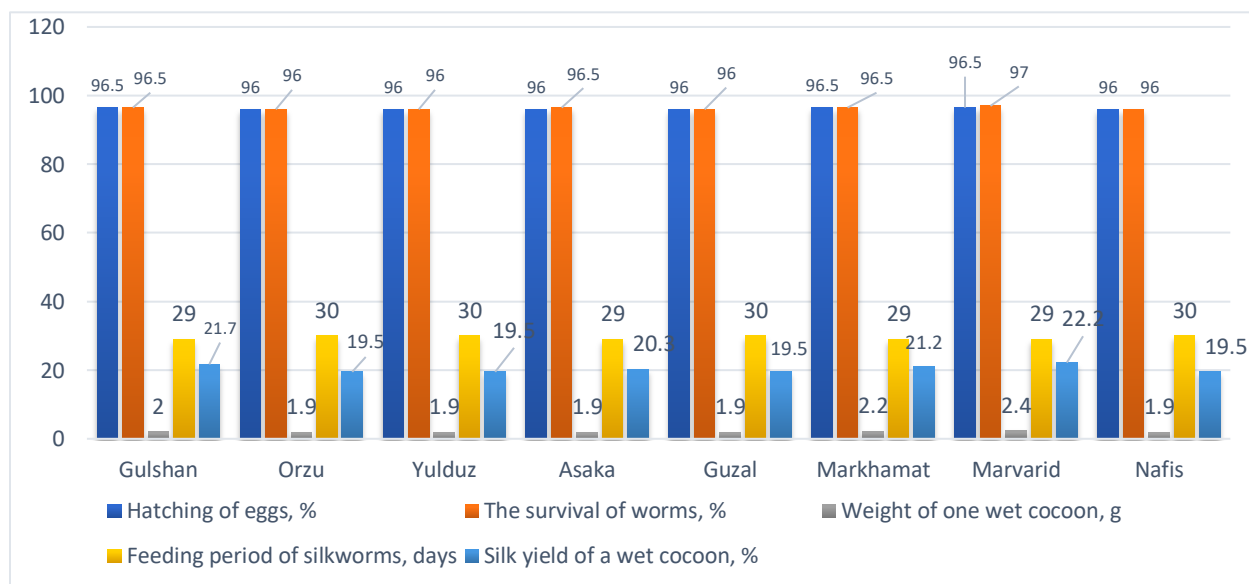
Here:  $df$ -degree of freedom,  $n$ -number of samples

### 3. Results and Discussion

The experimental results indicate that adaptation to local conditions significantly affects productivity. The biological indicators of the breeds in the experiment were determined. The biological indicators of the breeds brought from Uzbekistan are given in Table 1 and Figure 2.

**Table 1.** Biological and economic indicators of mulberry worm breeds imported from Uzbekistan.

S.N	Name of breed	Hatching of eggs, %	The survival of worms, %	Weight of one wet cocoon, g	Feeding period of silkworms, days	Silk yield of a wet cocoon, %
1	Gulshan	96.5	96.5	2.0	29	21.7
2	Orzu	96.0	96.0	1.9	30	19.5
3	Yulduz	96.0	96.0	1.9	30	19.5
4	Asaka	96.0	96.5	1.9	29	20.3
5	Guzal	96.0	96.0	1.9	30	19.5
6	Markhamat	96.5	96.5	2.2	29	21.2
7	Marvarid	96.5	97.0	2.4	29	22.2
8	Nafis	96.0	96.0	1.9	30	19.5



**Figure 2.** Various biological and economic indicators of mulberry silkworm breeds.

Table 1 shows the results of the study of 5 main biological and economic indicators of 8 breeds imported from Uzbekistan in the Sericulture Laboratory of the Sheki Regional Scientific Center of ANAS. It is clear from the table that the highest indicator for the revival of mulberry silkworm seeds was 96.5% in the Gulshan, Markhamat, and Marvarid breeds. However, it was relatively low in the Orzu, Nafis, Yulduz, Asaka, and Guzal breeds, 96.0%.

Viability is a key indicator directly affecting productivity and yield quality. If the viability of mulberry silkworms is low, the yield and quality will decrease. Among the breeds imported from Uzbekistan, the highest indicator for the viability of mulberry silkworms was 96.5 - 97.0% in the Marvarid, Markhamat, Asaka, and Gulshan breeds.

However, a relatively low indicator among the breeds was observed in the Orzu, Yulduz, Guzal, and Nafis breeds, 96%.

Wet cocoon weight is an important productivity indicator. Among the breeds in the experiment, the highest indicator in terms of cocoon weight was 2.0-2.4 g in the Marvarid, Gulshan, and Markhamat breeds. However, it was 1.9 g in the Orzu, Yulduz, Asaka, Nafis, and Guzal breeds.

The feeding period is a significant factor influencing production cost. It is clear from the table that the feeding period was 29 days in the Gulshan, Asaka, Marvarid, and Markhamat. Guzal and Marvarid breeds in the

experiment. However, during the research work, it was determined that the feeding period in the Orzu, Yulduz, Guzal, and Nafis breeds was relatively long, 30 days.

The silkiness of the wet cocoon was determined among the breeds imported from Uzbekistan. As a result of the research work, it was determined that in the Marvarid, Markhamat, Asaka, and Gulshan breeds, it was 20.3-22.2%. However, the silkiness of the wet cocoon in the Orzu, Yulduz, Nafis, and Guzal breeds was -19.5%.

Overall analysis shows that the Marvarid breed demonstrates superior performance across key productivity traits. Therefore, cocoon weight and silkiness should be considered primary criteria in breeding programs. The Markhamat breed also dominates in terms of productivity and quality. Gulshan has high viability and silkiness, but its cocoon weight is slightly lower. Other breeds - Orzu, Yulduz, Guzal, and Nafis, although good in terms of viability, are average in terms of cocoon weight and silkiness.

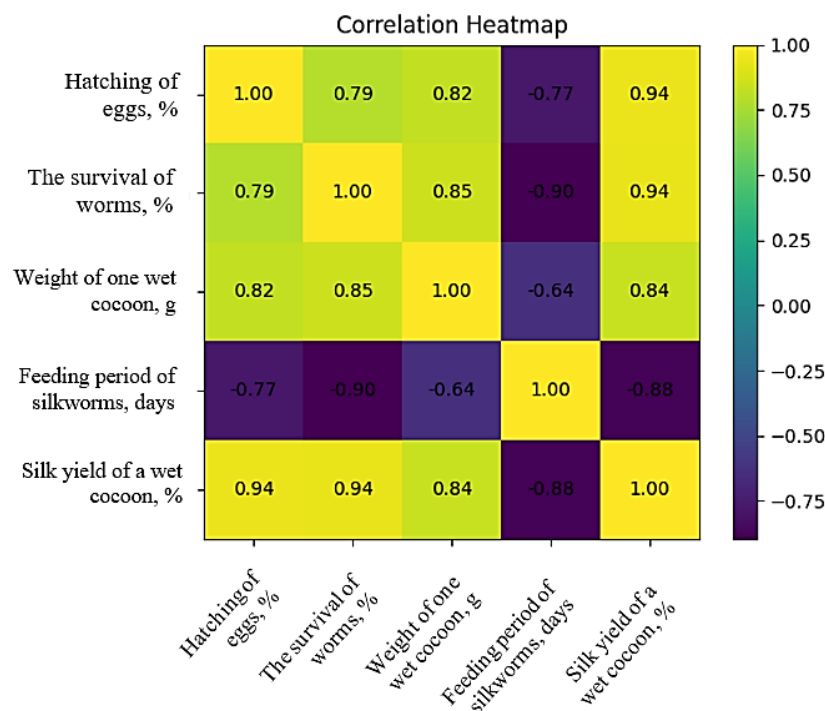
As a result, the main criteria in selection and breeding should be cocoon weight and silkiness; therefore, the Marvarid and Markhamat breeds should be selected as a priority.

In order to determine the mutual influence of various indicators and the strength of their relationship with each other, correlation coefficients between traits were calculated using the SPSS program, and the results are shown in Table 2 and a heatmap (Figure 3).

**Table 2.** Correlation coefficient between biological and economic indicators.

Indicator	Hatching of eggs, %	The survival of worms, %	Weight of one wet cocoon, g	Feeding period of silkworms, days	Silk yield of a wet cocoon, %
Hatching of eggs, %	1	0.788*	0.824*	-0.775*	0.941**
The survival of worms, %		1	0.853**	-0.898**	0.937**
Weight of one wet cocoon, g			1	-0.638	0.842**
Feeding period of silkworms, days				1	-0.881**
Silk yield of a wet cocoon, %					1

Note: \* and \*\* are statistically significant at 5% and 1% probability.



**Figure 3.** Heatmap for correlation between indicators.

Correlation analysis revealed relationships of varying strength and direction among the studied traits.

As a result of the conducted correlation analysis, it was determined that there are relationships of different directions and different strengths between the main biological and productivity indicators studied in the silkworm species. The results obtained show that there is a positive and statistically significant relationship ( $r=0.788$ ;  $p<0.05$ ) between the revival rate and viability. This confirms that individuals with a high revival rate also show higher viability.

Positive and reliable relationships were also determined between the revival rate and wet cocoon weight ( $r=0.824$ ;  $p<0.05$ ) and silkiness ( $r=0.941$ ;  $p<0.01$ ). In particular, the very strong correlation with silkiness indicates that this indicator is one of the main indicators in terms of productivity. At the same time, a negative relationship ( $r=-0.775$ ;  $p<0.05$ ) was observed between the revival rate and feeding period, which indicates that an increase in feeding period has a negative effect on revival indicators.

The relationships between viability and other parameters are also noteworthy. Thus, there are strong positive relationships between viability and cocoon weight ( $r=0.853$ ;  $p<0.01$ ) and silkiness ( $r=0.937$ ;  $p<0.01$ ). These results indicate that individuals with higher viability produce cocoons with heavier and higher silk yield. In addition, a strong negative relationship was observed between viability and feeding time ( $r=-0.898$ ;  $p<0.01$ ), which indicates that the extension of the development time negatively affects the overall sustainability of the organism. The strong positive relationship found between cocoon weight and silkiness ( $r=0.842$ ;  $p<0.01$ ) indicates that these two parameters are closely related to each other and confirms the possibility of their complex selection. On the other hand, a negative but not statistically significant relationship ( $r=-0.638$ ;  $p>0.05$ ) was found between cocoon weight and feeding period, which indicates that this relationship may be of a random nature.

A strong negative relationship ( $r=-0.881$ ;  $p<0.01$ ) was observed between the feeding period and silkiness. This result indicates that an increase in the feeding period leads to a decrease in silk productivity. Overall, the results obtained show that silkiness and viability act as the main indicators of productivity, and the feeding period is one of the factors that negatively affects these indicators.

#### **4. Conclusion**

The study demonstrates that biological and productivity indicators differ among the studied breeds imported from Uzbekistan. The Marvarid and Markhamat breeds are distinguished by high productivity and silk quality, while the Gulshan breed has a high survival rate and silkiness, but its cocoon weight is slightly lower. Other breeds - Orzu, Yulduz, Guzal, and Nafis - show satisfactory results in terms of viability, but are average in terms of cocoon weight and silkiness.

Correlation analysis showed that there are strong positive and statistically significant relationships between survival rate and viability, cocoon weight, and silkiness. At the same time, feeding time has a negative effect on these indicators. The strong positive correlation observed between cocoon weight and silkiness confirms the importance of a comprehensive assessment of these two indicators in the selection process.

Thus, cocoon weight and silkiness should be prioritized, and the Marvarid and Markhamat breeds are recommended for breeding.

#### **5. Suggestions**

Silkiness and viability should be prioritized in breeding programs.

1. Silkiness and viability should be given priority in breeding programs.
2. Technological and management measures should be implemented to shorten and optimize the feeding period.
3. Complex selection approaches covering several traits should be applied in order to increase productivity.

Thus, these strategies can contribute to increasing productivity and silk yield in silkworm breeding.

## Author Contributions

Gudurat M. Bakirov, Ramiz G. Bakirli, and Dursun M. Adigozalova conducted the experiments and performed the research. Safarli Kh. Khuzhamatov provided the species/materials. Ayaz M. Mammadov performed the statistical analysis, prepared the graphs and statistical data, and reviewed the manuscript.

## Conflict of Interest

The authors declare no conflicts of interest.

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Research Article

# Bioremediation Potential of Indigenous Earthworms in Radionuclide-Contaminated Soils of the Absheron Peninsula

Aygun S. Suleymanova<sup>1</sup>✉  and Constantin Daniel Negut<sup>2</sup>✉ 

<sup>1</sup>Department of Natural Sciences, School of Advanced Technologies and Innovation Engineering, Western Caspian University, 17 A, Ahmad Rajabli Street, III Parallel, AZ1072 Baku, Azerbaijan

<sup>2</sup>"Horia Hulubei" National Institute for R&D in Physics and Nuclear Engineering, 077125 Magurele, Romania

## Abstract

The current investigation illustrates the impact of radionuclide-contaminated soils from the Surakhani oil and gas processing plant and the Ramana iodine plant territories of the Absheron peninsula on the survival, feeding, and excretion functions of earthworms, as well as the antioxidant enzyme activity of the soils. Earthworms are highly sensitive to high radioactivity and are well-suited for assessing radioactive soils as bioindicators, as evidenced by model experiments. However, at lower contamination levels, earthworms accumulated radionuclides and contributed to their immobilisation in coprolites, potentially reducing their bioavailability. Contamination resulted in a significant increase ( $p < 0.05$ ) in catalase activity, suggesting the presence of oxidative stress responses. These discoveries underscore the dual function of earthworms as highly sensitive bioindicators and prospective agents for the bioremediation of radionuclide-contaminated soils.

**Keywords:** Absheron Peninsula, bioindicators, bioremediation, catalase activity, earthworms, soil contamination, radionuclides

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## 1. Introduction

Environmentally safe utilization of originally different organic wastes, as well as the remediation of anthropogenically contaminated soils, is the most actual problem nowadays. Mainly, the transformation of radionuclides from organic residues to the mineral fraction of soil and consequently into plants decreases only after deep ploughing of soils. In more than 1480 kKi/km<sup>2</sup> contaminated territories, ploughing is lethal for saprophyte microflora of soil and results in a decrease in biomass of some kinds of invertebrates in soils, and finally sharp pathological deformation of soils, which results in the acceleration of mineralization of isotopes [1]. For the fertility of soils, first, it is important to rehabilitate the soil organisms, microorganisms, and plants. During millions of years, earthworms have played a special part in the formation of the fertile layer of soil [2]. In normal terrestrial conditions, lumbricides make up 80% of the biomass of invertebrates in pedoecosystems [3], [4]. Hence, the International Commission of Radiological Protection requested the determination of the effect of radiation on non-human biota for the radioecological safety of the environment (ICRP 2003) and suggested earthworms as reference organisms for radiation protection of the environment [5], [6], [7], [8].

As a result of many years of oil production, large territories of the Absheron peninsula were contaminated with oil and sewage waters. Due to the leakage of residues of oil production, some radionuclides are transported to sewage waters, then to artificial canals and lakes, and radionuclides accumulated to soil and increase the radiation background of nearby areas. As well as some territories of the Absheron peninsula were directly contaminated with radionuclides and heavy metals due to residues of iodine production [9].

The study evaluates the response of native earthworms (*Nicodrilus caliginosus trapezoides* (Savigny)) to radionuclide-contaminated soils from Surakhani and Ramana regions. The effects on survival, feeding, excretion, and soil catalase activity were assessed under controlled laboratory conditions [10], [11].

## 2. Materials and Methods

Earthworms (*Nicodrilus caliginosus trapezoides* (Savigny)) have been collected from the grey-brown soils of the natural cenosis of the Absheron peninsula and prepared according to OECD standards [5]. Because of the high radioactivity, the soils of the territory Surakhani oil production plant were diluted in a 1:1 ratio, and the soil from the territory of Ramana iodine production plant in 1%, 2.5%, 5%, 10% concentrations with grey-brown soils. Earthworms with plant residues (for feeding) for 4 weeks by keeping 60-70% of humidity in the container, at room temperature, in laboratory conditions, have been cultivated in plastic dishes (with 300 ml volume) with pores for aeration. During the experiment for keeping a normal humidity level, the soils were 3 times watered with 20ml of distilled water. Radioactive background was measured with a radiometer-dosimeter (Inspector 1000, MKS AT 1125), and the activity of radionuclides was measured with a gamma spectrometer (HP Ge Gamma Spectrometer). Earthworms (*N. caliginosus trapezoides*) were collected from grey-brown soils of natural ecosystems of the Absheron Peninsula and prepared according to OECD Guideline 207 for earthworm acute toxicity testing. Due to high contamination levels, Surakhani soils were diluted at a 1:1 ratio with uncontaminated grey-brown soils. Ramana soils were tested at concentrations of 1%, 2.5%, 5%, and 10%.

Earthworms were maintained for four weeks in plastic containers (300 mL) with aeration pores under laboratory conditions at room temperature and 60-70% soil moisture. Plant residues were provided as a food source. Soil moisture was maintained by adding 20 mL of distilled water three times during the experiment.

The radioactive background was measured using a radiometer-dosimeter (Inspector 1000, MKS AT 1125), while radionuclide activity was determined using a gamma spectrometer (HPGe Gamma Spectrometer).

## 3. Statistical Analysis

Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to evaluate differences between control and treatment groups. Data are presented as mean  $\pm$  standard deviation (SD). Differences were considered statistically significant at  $p < 0.05$ .

## 4. Results

### 4.1. Survival of Earthworms

The effect of different concentrations of radioactive soils on the survival rate of earthworms is shown in Figure 1. It is obvious from that, with the increasing of radioactive concentration of soils, the survival rate decreases. In Surakhani soil samples, the final survival rate of earthworms was 13.30% less than in control (grey-brown soils) samples, whereas in 1% contaminated Ramana soils, this index decreased 5 times.

Survival decreased significantly with increasing radionuclide concentration ( $p < 0.05$ ). High-dose Ramana soils ( $\geq 2.5\%$ ) resulted in acute mortality within 72 hours. A statistically significant decrease in survival was observed with increasing radionuclide concentration ( $p < 0.05$ ) (Figure 1).

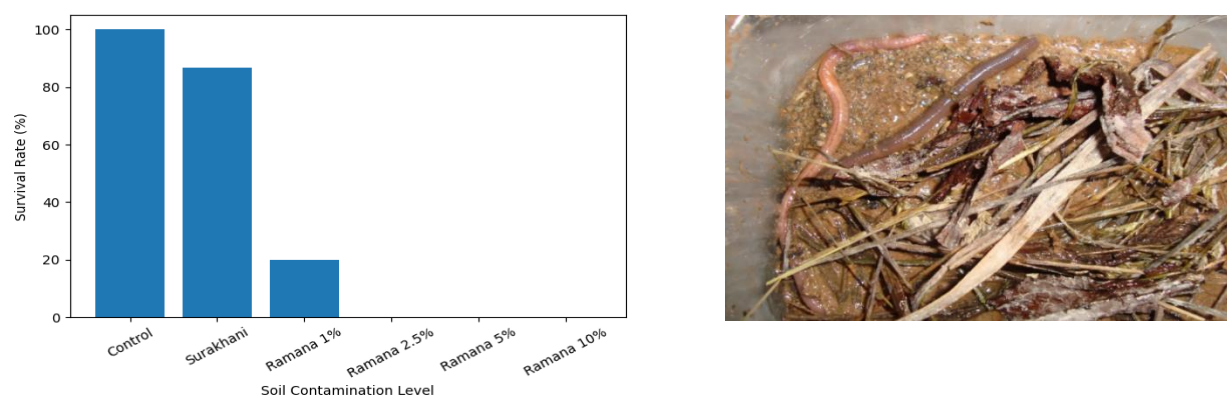


Figure 1. Survival rate of experimental earthworms.

#### 4.2. Feeding and Excretion Activity

The physiological analyses of earthworms in radionuclide-contaminated soils have shown that, with the increasing of radioactive concentration of radionuclides, the feeding and excretion of earthworms have been contrarily decreased (Figure 2, a & b). Thus, in control, non-contaminated soils, the feeding activity was 1.709g of initial 2g, and excretion activity was 9.2g per month; in Surakhani soils, these data was 34% less feeding activity than in control, and excretion activity was about 27 % less. For Ramana soils, it appeared as 24% less feeding and 29% less excretion than in control, but for 1% contaminated Ramana soils, which was 20% less feeding and 89% less excretion activity than in control samples for 2.5% contaminated soils.

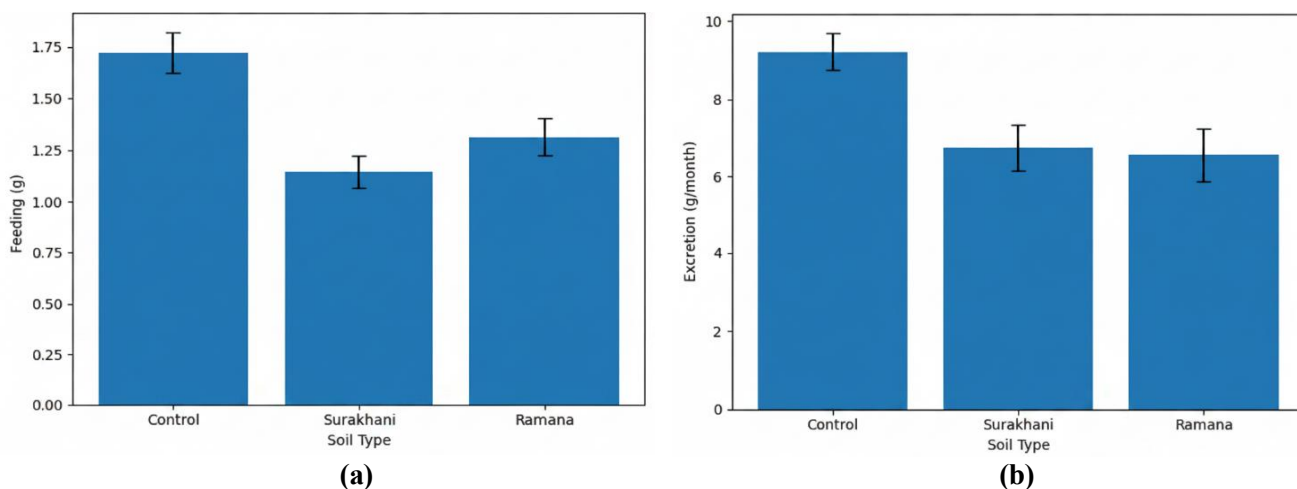
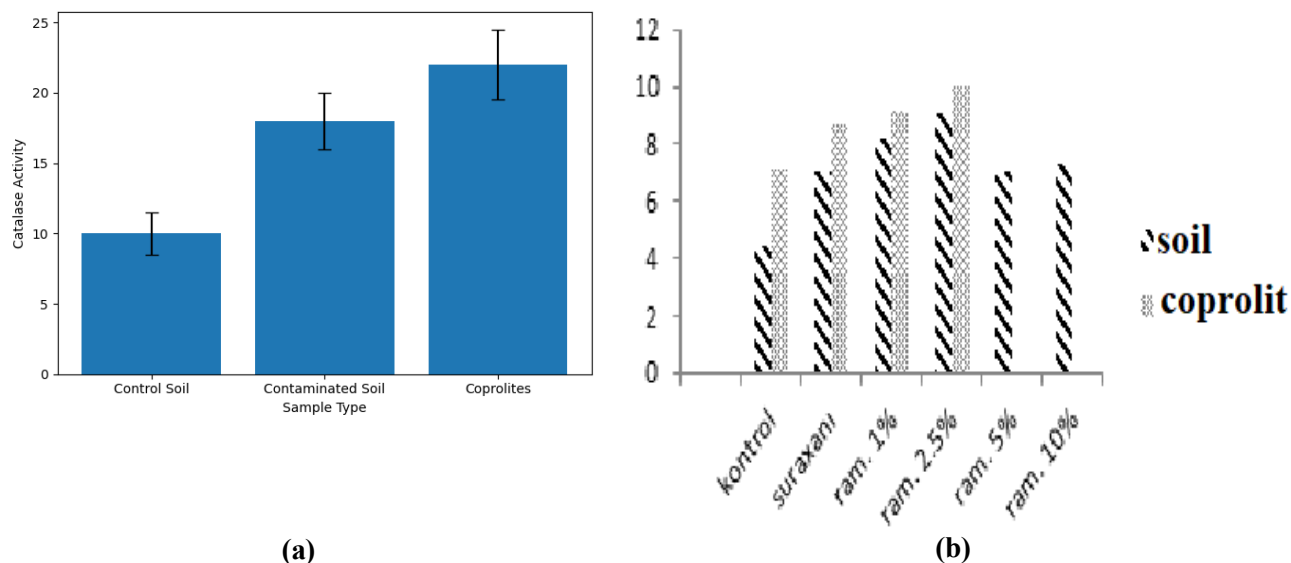


Figure 2. (a) Feeding activity (Mean  $\pm$ SD) and (b) Excretion activity (Mean  $\pm$ SD).

Excretion activity was more sensitive to contamination than feeding, indicating early physiological disruption.

#### 4.3. Catalase Activity

At the end of the month, catalase activity was measured in the earthworm excreta and in the rest of the experimental soils. It was established that, following the radioactive effects of radionuclides on earthworms and soil biota, catalase activity increased in a direct proportion to radionuclide concentration (Figure 3, a & b). It was determined that in coprolites (excrements of earthworms), the quantity of catalase was a little bit more than in the remaining soils of the samples. This interesting difference is due to the action of saprotrophic bacteria, which symbiotically live in the gut of earthworms, so their action under the radioactive soils that pass through the intestines of earthworms also increases the catalase activity.



**Figure 3.** (a) Catalase activity (Mean  $\pm$ SD) and (b) The quantity of catalase in experimental soils and coprolites of earthworms ( $\text{cm}^3 \text{O}_2/\text{g}$  2min).

The observed increase in catalase activity reflects enhanced oxidative stress due to radionuclide exposure.

#### 4.4. Radionuclide Accumulation

One of the significant points of experiments is the accumulation of radionuclides by earthworms (Table 1). It was determined that earthworms, by taking the soil through their gut, absorb more than 50% of radionuclides, absorbing them in coprolites as stable chelate complexes. So, radionuclides isolated from soil are becoming non-toxic for plants. Earthworms significantly altered radionuclide distribution between soil and coprolites ( $p < 0.05$ ).

**Table 1.** Quantity of radionuclides before and after experiments in model soil samples from Surakhani and Ramana regions (Bq/kg).

Samples	Radionuclide compounds of soils					
	$^{137}\text{Cs}$	$^{40}\text{K}$	$^{226}\text{Ra}$	$^{228}\text{Ra}$	$^{235}\text{U}$	$^{238}\text{U}$
Control Grey brown	-	-	$16 \pm 2$	$15 \pm 2$	$2.5 \pm 0.3$	$123 \pm 12$
<b>Surakhani soils</b>						
Initial soils	$10,7 \pm 0,8$	$133,5 \pm 5,5$	$1004 \pm 5$	$14,7 \pm 1,1$	-	-
Remained soils	-	$389 \pm 33$	$732 \pm 6$	$22 \pm 2$	-	-
Coprolite	-	$561 \pm 33$	$709 \pm 7,7$	$8,7 \pm 0,4$	-	-
<b>Ramana soils</b>						
Initial soils	-	-	$35345 \pm 98$	$436 \pm 27$	$622 \pm 15$	$30400 \pm 800$
<b>Remained soils</b>						
1%	-	-	$560 \pm 56$	$23 \pm 2,3$	$11 \pm 1,7$	$542 \pm 53$
2,5%	-	-	$1055 \pm 103$	$22,7 \pm 3$	$10,7 \pm 2$	$512 \pm 49$
5%	-	-	$2170 \pm 223$	$33 \pm 3$	$28 \pm 2,5$	$1351 \pm 107$
10%	-	-	$4140 \pm 413$	$69 \pm 6,7$	$44,7 \pm 4,7$	$2177 \pm 220$
<b>Coprolites</b>						
1%	-	-	$200 \pm 10$	$25 \pm 3$	$30 \pm 5$	$1460 \pm 100$
2,5%	-	-	$100 \pm 10$	$6,0 \pm 0,7$	$60 \pm 7$	$2900 \pm 150$
5%	-	-	$780 \pm 30$	$40 \pm 10$	$140 \pm 15$	$6880 \pm 150$
10%	-	-	-	-	-	-

## **5. Discussion**

The results of this investigation are in accordance with earlier studies showing that soil invertebrates are extremely vulnerable to radiation-induced stress. A well-established mechanism under radiation stress is the activation of antioxidant defence systems in response to elevated levels of reactive oxygen species (ROS), as shown by the observed increase in catalase activity. Similar enzymatic reactions have been reported in contaminated environments, emphasising the significance of oxidative stress as a major toxicity mechanism. Coprolites' increased enzymatic activity raises the possibility that gut-associated bacteria could improve detoxification procedures.

The detrimental effects of radioactive exposure on soil invertebrates are confirmed by the reported decrease in survival and physiological activity. Studies on radiation-induced stress in soil ecosystems, where oxidative damage is a major factor, have revealed similar results.

Additionally, earthworms' capacity to store radionuclides and transfer them into coprolites suggests a possible natural detoxifying process. This process may assist the use of radionuclides in bioremediation techniques by lowering their bioavailability and limiting their transfer into plants and higher trophic levels. This is consistent with other research showing how earthworms may immobilise contaminants and heavy metals. Nevertheless, the efficacy of this mechanism seems to be concentration-dependent, as acute mortality was observed in the presence of elevated contamination levels.

The results suggest that earthworms have the potential to be used in sustainable soil remediation strategies, particularly in moderately contaminated environments.

## **6. Conclusions**

This research supports the concept that soil invertebrates are exceedingly susceptible to radiation-induced stress, as elevated radionuclide exposure results in substantial toxicity, including diminished physiological activity and survival. The observed increase in catalase activity suggests that earthworms initiate antioxidant defence mechanisms to combat oxidative damage (ROS), which is a consequence of this stress.

Key discoveries concerning detoxification mechanisms indicate that earthworms accumulate radionuclides and recirculate them as coprolites (excrement). This implies a natural immobilisation mechanism that diminishes the bioavailability of these contaminants. The significant function of gut-associated microbiota in detoxification processes is indicated by the increased enzymatic activity of these coprolites.

Nevertheless, the efficacy of this mechanism is contingent upon the concentration, as acute mortality was observed in the presence of elevated contamination levels. Consequently, earthworms are most effectively employed as a sustainable, long-term bioremediation strategy (vermediation) in moderately contaminated environments, as opposed to severely contaminated sites.

Earthworms are very sensitive bioindicators of radiation pollution. Under conditions of radiation stress, a significant decrease in their physiological functions is observed. The increase in catalase activity confirms the occurrence of oxidative stress mechanisms. At the same time, worms have the ability to accumulate radionuclides and immobilize them to a certain extent. These properties make them possible to use as effective bioremediation on agents in low and moderate levels of contamination.

## **Author Contributions**

Aygun S. Suleymanova contributed to the experimental investigation and the writing of the manuscript. Constantin Daniel Negut performed the gamma spectrometric measurements and reviewed the manuscript.

## **Conflict of Interest**

The authors declare no conflicts of interest.

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Review Article

# The Role of Chemistry in the Development of Agriculture

Nizami A. Akbarov<sup>1</sup>  and Kamala O. Iskandarova<sup>2</sup> 

<sup>1</sup>Department of Analytical and Organic Chemistry, Azerbaijan State Pedagogical University, Uzeyir Hajibeyli 68, Sabail District, AZ1000 Baku, Azerbaijan

<sup>2</sup>Department of General Chemistry and Chemistry Teaching Technology, Azerbaijan State Pedagogical University, Uzeyir Hajibeyli 68, Sabail District, AZ1000 Baku, Azerbaijan

## Abstract

This article analyzes the role of chemistry in agriculture in detail and particularly emphasizes the importance of chemical fertilizers in increasing crop productivity. The article extensively explains the chemical composition, structural properties, and behavior of fertilizers in the soil-plant system. At the same time, the classification of fertilizers - simple and complex fertilizers, as well as the main macroelements - nitrogen, phosphorus, and potassium fertilizers- is analyzed in separate sections, showing their functions in plant development and application characteristics. In addition, the article provides a scientific explanation of fertilizer production technologies, their effects on soil fertility, and the physiological processes of plants. Plant protection products are also examined, explaining the role of pesticides, herbicides, and other protective agents in maintaining productivity and combating diseases. Consequently, the application of chemistry in agriculture is shown to be one of the key factors for sustainable and efficient production.

**Keywords:** chemical preparations, agriculture, nutrients, macrofertilizers, microfertilizers, mineral fertilizers

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## 1. Introduction

As the science of chemistry develops, its influence is constantly felt in the multifaceted processes of human life. It is very difficult to imagine life without the application of chemical science's achievements. In particular, the role of chemistry in the development of agriculture is crucial. Thus, the production of various mineral fertilizers significantly increases the productivity of plants. Some chemical preparations are successfully used against plant pests [1], [2].

## 2. Characteristics and Classification of Fertilizers

When the soil lacks nutrients essential for plant nutrition and growth, mainly nitrogen, phosphorus, and potassium, these elements are added to the soil artificially in the form of mineral fertilizers. The use of mineral fertilizers helps increase productivity.

Plants contain more than 70 different chemical elements. However, among these elements, 16 are considered essential for the normal growth and development of plants. Some of these elements are obtained through photosynthesis, while others are absorbed from the soil. Elements necessary for the normal development of plants are called nutrients. There are three main nutrients: nitrogen, phosphorus, and potassium [3].

Fertilizers are substances that contain nutrients. Mineral fertilizers are compounds, mainly salts, that contain nutrients for plants.

Elements required by plants in large quantities (C, O, H, N, P, K, Ca, Mg, Fe, S) are defined as macroelements, and fertilizers containing these elements are referred to as macrofertilizers (or conventional fertilizers).

Elements required by plants in small amounts (B, Cu, Co, Mn, Zn, Mo, etc.) are defined as microelements, and fertilizers containing these elements are known as microfertilizers. [4].

Of the macronutrients, plants need three elements in greater quantities, such as nitrogen, phosphorus, and potassium. These are called essential nutrients.

The classification of mineral fertilizers can also be expressed as follows (Table 1) [5], [6]:

**Table 1.** Classification of mineral fertilizers

According to their main nutrients	According to their composition	According to their origin	According to their aggregate state
Nitrogen fertilizers	Simple fertilizers	Mineral fertilizers (fertilizers mentioned above)	Solid fertilizers (salts, phosphates)
Phosphorus fertilizers	Compound / complex fertilizers	Organic fertilizers (manure, poultry droppings, etc.)	Liquid fertilizers (liquid NH <sub>3</sub> , ammonia water)
Potassium fertilizers	Mixed fertilizers		

Fertilizers that contain only one nutrient are called simple fertilizers. For example: KCl, NaNO<sub>3</sub>, NH<sub>4</sub>NO<sub>3</sub>, Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>, CaHPO<sub>4</sub>·H<sub>2</sub>O, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, and CO(NH<sub>2</sub>)<sub>2</sub>.

Fertilizers that contain two or three nutrients are called compound fertilizers. For example: KNO<sub>3</sub>, (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, and NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>.



Mixed fertilizers contain different types, such as simple, complex fertilizers, or both:

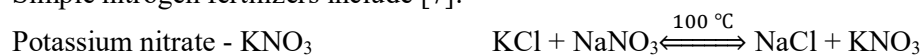


Nitrophoska is a mixture of three salts: (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> + NH<sub>4</sub>NO<sub>3</sub> + KCl

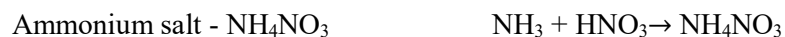
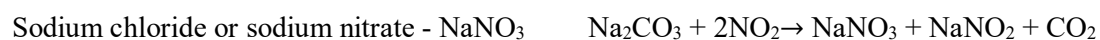
### 3. Nitrogen Fertilizers

Nitrogen fertilizers are fertilizers that contain the nutrient element nitrogen. A lack of nitrogen in plants makes it difficult to form green mass, plants develop poorly, and their leaves turn yellow. Nitrogen plays a special role in plant nutrition. It is included in the composition of chlorophyll and proteins. Plants absorb nitrogen mainly in the form of ammonium NH<sub>4</sub><sup>+</sup> and nitrate NO<sub>3</sub><sup>-</sup> ions.

Simple nitrogen fertilizers include [7]:



The salts obtained from this reaction are highly soluble in water. However, at 100°C, sodium chloride is less soluble than potassium nitrate. At this point, sodium chloride crystallizes in the solid solution, and the equilibrium in this reversible reaction shifts to the right [8], [9], [10].



In addition to those mentioned, simple nitrogen fertilizers include calcium salt (Ca(NO<sub>3</sub>)<sub>2</sub>), ammonium sulfate (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, liquid ammonia, and ammonia water. NaNO<sub>3</sub>, KNO<sub>3</sub>, NH<sub>4</sub>NO<sub>3</sub>, and (Ca(NO<sub>3</sub>)<sub>2</sub>) are called salts.

Calculation of the nutritional value of nitrogen fertilizers is expressed as the mass fraction of nitrogen in the same substance and shown as a percentage. The higher the mass fraction of nitrogen in a fertilizer, the more nutritious it is or the richer it is in nitrogen. For instance, the nutritional value of nitrogen in urea is calculated as follows:

$$\omega(\text{N}), \% = \frac{A_r(\text{N}) \cdot 2}{M_r[\text{CO}(\text{NH}_2)_2]} \cdot 100\% = \frac{28}{60} \cdot 100\% = 46,7\%$$

When excess nitrates are added to the soil, they accumulate in plants. Such plants harm human health when used as food.

Examples of complex fertilizers containing nitrogen include ammonium dihydroorthophosphate (ammophos)  $\text{NH}_4\text{H}_2\text{PO}_4$ , ammonium hydroorthophosphate (diammophos)  $(\text{NH}_4)_2\text{HPO}_4$ , potassium nitrate  $\text{KNO}_3$ , etc.

In the soil, fertilizers are converted into a form that plants can absorb:

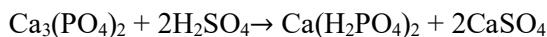


In addition to mineral nitrogen fertilizers, organic nitrogen fertilizers (manure, poultry droppings, etc.) are also used [11].

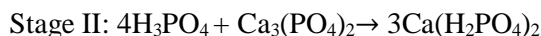
#### 4. Phosphorus Fertilizers

Phosphorus plays a major role in the growth of flowers and fruits of plants. Phosphorus fertilizers are the following (the main raw material for the production of phosphorus fertilizers is phosphorite -  $\text{Ca}_3(\text{PO}_4)_2$ ):

1. Phosphorite flour ( $\text{Ca}_3(\text{PO}_4)_2$ ) is obtained by finely grinding phosphorites. Phosphorites are poorly soluble in water but well soluble in organic acids. This fertilizer is usually applied to acidic soils.
2. Simple superphosphate is obtained from the reaction of  $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot 2\text{CaSO}_4$  phosphorite ( $\text{Ca}_3(\text{PO}_4)_2$ ) or apatite with sulfuric acid:

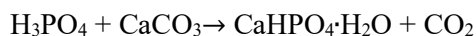
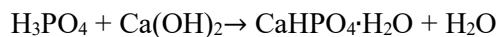


3. Double superphosphate  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  is obtained in two stages. At this time,  $\text{CaSO}_4$  is formed as an intermediate product:



The  $\text{CaSO}_4$  obtained in the first stage precipitates and is filtered and separated.

4. The precipitate  $\text{CaHPO}_4 \cdot \text{H}_2\text{O}$  is obtained from the interaction of orthophosphoric acid with calcium hydroxide and calcium carbonate (limestone):



5. Bone meal consists mainly of  $\text{Ca}_3(\text{PO}_4)_2$  and is obtained by grinding the bones of domestic animals [12].

#### 5. Potassium Fertilizers

Potassium also plays a significant role in plant physiology. Thus, they accelerate the photosynthesis process, help accumulate carbohydrates, and strengthen the stems of cereal plants. Potassium fertilizers are also found in nature. Potassium fertilizers are:

- Sylvite mineral -  $\text{KCl}$
- Sylvinit mineral -  $\text{KCl} \cdot \text{NaCl}$

- Kainite mineral -  $\text{KCl} \cdot \text{MgSO}_4 \cdot 3\text{H}_2\text{O}$
- Carnallite mineral -  $\text{KCl} \cdot \text{MgCl}_2 \cdot 6\text{H}_2\text{O}$

## 6. Nutritional Value of Fertilizers

In nitrogen fertilizers, the nutritional value of the fertilizer is expressed as the mass fraction of nitrogen in the nitrogenous compound considered as fertilizer ( $\omega\%$  N):

$$\text{CO}(\text{NH}_2)_2 \quad \omega_{\text{N}} = \frac{\text{Ar}(\text{N}) \cdot 2}{\text{Mr}(\text{CO}(\text{NH}_2)_2)} \cdot 100$$

In phosphorus fertilizers, the nutritional value of the fertilizer is calculated based on  $\text{P}_2\text{O}_5$ , which corresponds to the phosphorus in the phosphorus compound considered as fertilizer:

$$\text{Ca}(\text{H}_2\text{PO}_4)_2 \quad \omega_{\text{P}_2\text{O}_5} = \frac{\text{Mr}(\text{P}_2\text{O}_5)}{\text{Mr}(\text{Ca}(\text{H}_2\text{PO}_4)_2)} \cdot 100$$

$$\text{CaHPO}_4 \cdot \text{H}_2\text{O} \quad \omega_{\text{P}_2\text{O}_5} = \frac{\text{Mr}(\text{P}_2\text{O}_5)}{\text{Mr}(\text{CaHPO}_4 \cdot \text{H}_2\text{O}) \cdot 2} \cdot 100$$

The reason for taking twice the molar mass of the fertilizer in the denominator of the second equation is that the  $\text{P}_2\text{O}_5$  molecule in the figure contains 2 moles of P atoms. That is, 1 mole of  $\text{P}_2\text{O}_5$  corresponds to 2 moles of  $\text{CaHPO}_4 \cdot \text{H}_2\text{O}$ .

In potash fertilizers, the nutritional value of the fertilizer is also calculated based on the  $\text{K}_2\text{O}$ , which corresponds to the potassium in the potash compound considered as the fertilizer:

$$\text{KCl} \quad \omega_{\text{K}_2\text{O}} = \frac{\text{Mr}(\text{K}_2\text{O})}{\text{Mr}(\text{KCl}) \cdot 2} \cdot 100$$

$$\text{K}_2\text{CO}_3 \quad \omega_{\text{K}_2\text{O}} = \frac{\text{Mr}(\text{K}_2\text{O})}{\text{Mr}(\text{K}_2\text{CO}_3)} \cdot 100$$

Here, the reason for taking twice the molar mass of the fertilizer in the denominator of the first equation is that the  $\text{K}_2\text{O}$  molecule in the formula contains 2 moles of K atoms. That is, 1 mole of  $\text{K}_2\text{O}$  corresponds to 2 moles of KCl.

## 7. Plant Protection Products

Currently, more than a thousand crop protection products are available, which can be classified in various ways [13].

According to the type of pest:

- Insecticides: used against insects.
- Acaricides: used against ticks.
- Ovicides: used to destroy the oocysts (eggs) of insect pests, including mites.
- Larvicides: used to kill insects in the larval stage.
- Nematocides: used to kill roundworms (nematodes).
- Rodenticides: used to protect plants from mammals and rodents.
- Fungicides: used to combat fungal infections.

- Bactericides: used to destroy bacterial pathogens.
- Herbicides: used to control weeds and grasses.
- Arboricides: used only to kill trees or shrubs.
- Algae: used to control algae.
- Virocides: used to protect plants from viral diseases.

According to chemical composition:

- Inorganic (containing sulfur, fluorine, copper, mercury)
- Organic (organic phosphorus and organic chlorine compounds, nitrophenols, etc.)
- Biogenic

According to toxicity:

- Low toxicity
- Moderate toxicity
- High toxicity

## **8. Conclusion**

Plant protection products must be used with caution due to their potential toxicity. Improper use of crop protection products can cause serious environmental problems, including:

- *the emergence of persistent pest populations that will require more resources to control.*
- *potential to affect beneficial fauna and flora of ecosystems.*
- *the potential to interfere with biological and biogeochemical cycles in nature.*

Mineral fertilizers are applied when there is a lack of nutrients in the soil. Plant protection products are used to combat agricultural pests. It is crucial to follow the rules for applying mineral fertilizers and plant protection products to protect human and animal health and prevent environmental problems.

Therefore, mineral fertilizers and pesticides should be applied in controlled and recommended amounts to avoid ecological imbalance.

## **Author Contributions**

Both authors contributed substantially to the study and approved the submitted version.

## **Conflict of Interest**

The authors declare no conflicts of interest.

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Research Article

# Molecular Screening of *Ustilago maydis* Fungus in Maize Genotypes

Surayya T. Ismayilova<sup>1</sup>✉  and Saltanat A. Aghayeva<sup>1</sup>✉ 

<sup>1</sup>Department of Natural Sciences, School of Advanced Technologies and Innovation Engineering, Western Caspian University, 17 A, Ahmad Rajabli Street, III Parallel, AZ1072 Baku, Azerbaijan

## Abstract

In modern agriculture, protecting plants from diseases and pests is one of the main measures to increase their productivity and improve their quality. Maize plays a significant role in the food and livestock industries. However, various fungal diseases cause serious damage to productivity. In particular, the smut disease is distinguished by its serious damage, which is caused by the fungus *Ustilago maydis* of maize and is a widespread disease. This disease causes reduced yield and deterioration of grain quality. This study aimed to identify maize genotypes with complex resistance to this disease and to create collections of morphological, physiological, and economically valuable traits. This information plays an important role in the selection and conservation of disease-resistant variants of maize varieties. The study also provides a valuable source of molecular information to help prevent the spread of maize disease and strengthen genetic defenses. The study included field observations, laboratory diagnostics, assessment of the extent of infection, and selection of resistant samples. As a result, genotypes with different levels of resistance were identified, initial trait collections were formed, and promising lines were selected for future selection.

**Keywords:** maize, plant disease, *Mycosarcoma maydis*, stable genotypes, selection

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## 1. Introduction

Maize (*Zea mays* L.) is the most significant and productive cereal forage crop in the world's agricultural system. Its versatile use and high productivity distinguish this crop. As a cereal forage crop, maize is superior to all cereal forage crops in terms of productivity and feed value. Countries around the world use 20% of maize grain for food, 15-20% for technical purposes, and the remaining 2/3 as feed. Depending on soil-climatic conditions and cultivation technology, the grain contains 9-12% protein, 1-2% sugar, 4-8% fat (40% in the kernel), 65-70% nitrogen-free extractives, 1.5-2% ash elements, mineral salts, and vitamins. Flour, cereal, canned food, starch, ethyl alcohol, beer, glucose, sucrose, oil, glutamic acid, copper (Cu), and vitamins E and C are obtained from maize. Paper, linoleum, viscose, artificial cork, plastic, activated carbon, etc., are made from the stem, leaves, and stalks [1].

The widespread use of maize in food, feed, and technical purposes makes maintaining the health of this plant a strategic issue. The occurrence of diseases in cultivated fields leads not only to crop losses but also to increased production costs. Therefore, the study of the main diseases of maize and their spread characteristics is one of the urgent problems [1], [2].

Smut of maize is a disease caused by *Ustilago maydis* (DC.) Corda is widespread worldwide. High temperatures and low humidity create favorable conditions for the development of the disease. *U. maydis* is a parasitic, biotrophic fungus and plant pathogen that primarily infects plants of the cereal family. Infections caused by it can significantly reduce the yield of maize, wheat, barley, and other major crops [3], [4].

One of the main visible signs of *U. maydis* infection is the formation of large tumors on the ears of maize; severe infection can lead to complete loss of the ear and crop loss [4]. *U. maydis* does not cause immediate death of plant

cells upon infection; it gradually establishes a close symbiotic relationship with the infected plant, which allows the plant to survive throughout the fungus's entire life cycle [3], [5], [6].

Smut disease of maize causes pathological growths (swellings, tumors) on all parts of the plant, most commonly on the stem and shoots. Usually, the first lesions, blisters, form on the root collar, then on the leaves and stems, and later the panicles and pedicels [2]. When the leaves are infected, the blisters are observed as groups of striated wrinkles. Individual flowers on the brooms are infected, and small sac-shaped blisters form. Larger growths - blisters form on the stems and limbs. The diameter of such cysts sometimes reaches 30 cm. In the derivatives, cysts and teliospores of the fungus are formed. They germinate and, at this time, form dikaryotic mycelium or sporidia. The formation of blisters occurs over a period of about two weeks. The spores that develop in the blisters are capable of germination and can infect the plant throughout its entire growing season (Figure 1).



**Figure 1.** *Ustilago maydis* of maize. Both plates show infected grains with black tumor-like galls.

The fungus *U. maydis* cannot spread diffusely through the plant. Therefore, each blister forms only at the site of infection. Another characteristic of this pathogen is that it only infects vegetative cells. The mycelium can't reach the embryo, but it infects the pea pericarp. Young ovaries are atrophied when infected. In brooms, galls occur on the anthers and on the stigma. The anthers are also infected.

Dry teliospores can retain their viability for up to four years [2]. Under natural conditions, they quickly lose their ability to germinate because they are constantly wetted by rain and irrigation water. However, since the teliospores contained in sorghum tubers in the soil are poorly wetted by water, they are not destroyed in autumn, winter, and spring. When the soil is cultivated in spring (plowing, raking, hoeing), the sorghum tubers are scattered, and the spores are spread by the wind, causing the initial infection of maize plants. In very rare cases, teliospores that accidentally overwinter on the surface of the seed can be present as a primary infection.

The period from tillering to milk ripening is the period of high susceptibility of plants to the disease. During this period, maize plants are susceptible to the fungus *Ustilago maydis*, the causative agent of blister blight. This is also why blister beetles often spread as epiphytoses in various countries around the world.

The rate of development of powdery mildew depends on soil moisture. Plant infection is consistently high at soil moisture levels below and above 60%. The minimum temperature for teliospore germination is 0-5°C, and the optimum is 20-30°C [2]. The process of the formation of derivatives stops at temperatures below 21°C. Teliospores germinate better at higher temperatures. Spores can survive in the soil for up to 3 years. Late sowing also accelerates the infection of maize. Prolonged high humidity during the growing season of maize limits its infection with blister rust, while high temperatures and uneven moisture supply to plants increase infection. Dense plantings are also factors that stimulate the infection with blister rust.

The disease is more harmful when the maize stalks and pods are infected. In this case, yield is reduced by 25-30%, and the reduction also depends on the duration of the infection, which part of the plant is infected, and the number of blisters [2], [4].

Classification of the pathogen:

- Kingdom: Mycota
- Division: Basidiomycota
- Class: Basidiomycetes
- Order: Ustilaginales
- Family: Ustilaginaceae
- Genus: *Ustilago* (Pers.) Roussel
- Species name: *Ustilago maydis* (DC.) Corda

### **1.1. Genetic Resistance of Maize to Common Smut Disease**

Genetic resistance to maize blast is one of the most effective control methods in plant breeding [4]. Chemical control methods are expensive, have a negative impact on the ecosystem, and are not sustainable [3]. However, genetic resistance creates natural and long-term protection against disease by strengthening the plant's internal defense mechanisms. This approach ensures both agrobiological sustainability and allows for the maintenance of high yields. Genetically stable genotypes significantly reduce the level of infection in the field, minimize additional costs, and make the selection process more targeted. For this reason, identifying genotypes with complex resistance and including them in breeding programs is one of the main directions in modern plant breeding [4].

Smut is one of the diseases that causes serious crop losses in maize production worldwide, reducing both seed yield and overall plant quality. The risk of disease spread is particularly high in agricultural regions, and in favorable climatic conditions, the infection rate can reach 30-80%. Chemical control of this disease is ineffective and considered economically and ecologically unviable. Therefore, the identification of genotypes that exhibit complex resistance is of strategic importance both at the national and international levels.

The main objective of this study is to identify genotypes that exhibit complex resistance to maize blast disease and to create collections of morphological, physiological, and economically important traits based on these genotypes.

### **1.2. Molecular and Genetic Basis of Resistance to Common Smut in Maize**

Resistance of maize to common smut disease is a complex polygenic trait formed through the interaction of multiple genes [7], [8]. This resistance mechanism is associated with the plant's early defense responses, structural characteristics of the cell wall, and the activation of specific genes involved in pathogen defense. During plant-pathogen interactions, receptor proteins located on the plant cell surface initially recognize pathogen-derived signaling molecules and subsequently trigger defense signaling pathways [8]. As a result, the synthesis of reactive oxygen species increases, the expression of defense-related genes is enhanced, and reinforcement of the cell wall occurs.

During infection by *Ustilago maydis*, hormonal balance within maize cells also undergoes significant changes. In particular, the jasmonate and salicylic acid signaling pathways play essential roles in regulating defense responses. In resistant genotypes, these signaling pathways are activated more rapidly and in a coordinated manner. In susceptible genotypes, however, pathogen effector proteins may suppress plant defense mechanisms, leading to intensified tumor (gall) formation.

At the molecular level, one of the key mechanisms associated with resistance involves the degree of methyl-esterification of pectin substances present in the plant cell wall [7], [9]. A more stable cell wall structure restricts pathogen penetration into plant tissues. In addition, the accumulation of phenolic compounds and lignin also limits the spread of infection. These characteristics are typically observed at higher levels in resistant genotypes [7].

In recent years, marker-assisted selection methods have been widely applied for the identification of resistant genotypes [10], [11]. In particular, SSR, SNP, and other molecular markers enable the detection of loci associated with disease resistance [10]. Compared with phenotypic evaluation, this approach is more precise and efficient.

The molecular screening data obtained through such analyses facilitate the accurate selection of parental forms in breeding programs and contribute to the development of disease-resistant cultivars.

Thus, resistance of maize to common smut disease represents a complex trait formed at structural, physiological, and molecular levels. Comprehensive investigation of these mechanisms accelerates the identification of resistant genotypes and enables the development of long-term, environmentally sustainable strategies for disease management.

## 2. Materials and Methods

### 2.1. Characteristics of Research Materials

Researchers conducted experimental studies in Azerbaijan to identify genes for resistance to blister blast disease in maize genotypes, focusing on 15 maize genotypes that differ in disease resistance, architecture, and other physiological indicators. During the study, plant samples showing both healthy and disease symptoms were selected for analysis.

### 2.2. Isolation of Nuclear DNA from Maize Genotypes

DNA was isolated from plant samples by the CTAB method. Leaf particles cut from plants were crushed in liquid nitrogen and suspended in 1 ml of CTAB extraction buffer (100 mM Tris-HCl, pH 8.0; 20 mM EDTA, pH 8.0; 1.4 mM NaCl; 40 mM  $\beta$ -mercaptoethanol) heated to 60°C in a water bath.

The homogenate is continuously mixed in a vortex apparatus. Then 0.4 ml of chloroform (99.8%) is added to each test tube and mixed carefully. The test tubes are then placed in a water bath and incubated at 60°C for 10 minutes. After incubation, the test tubes are centrifuged in an Eppendorf benchtop centrifuge (1400 g) at room temperature for 10 min. The supernatant is then carefully transferred to clean 1.5 ml Eppendorf test tubes (precipitate should not be mixed with the filtered supernatant), and 0.6 ml of cold isopropanol is added, mixed carefully, and kept at room temperature for 3-5 min. The precipitate is washed several times in 70% ethyl alcohol, dried in a bench thermostat, and then dissolved in TE (10 mM Tris-HCl, pH 8; 1 mM EDTA) buffer. The samples are stored overnight in a refrigerator at 40°C to ensure complete dissolution of the DNA in the buffer.

### 2.3. Determination of DNA Purity and Optical Density

The purity of the separated DNA samples is checked by spectrophotometry (ULTROSPEC 3300 PRO, "AMERSHAM," USA). Thus, the optical density of the DNA samples at wavelengths of 260 and 280 nm is determined. If  $1.8 \leq [D_{260}/D_{280}] \leq 2$ , the separated DNA samples are considered suitable for further experiments; otherwise, the DNA sample separation should be repeated and carefully performed.

## 3. Results and Discussion

The study showed that genetic diversity exists in maize. (*Zea mays* L.) plays an important role in its response to *Ustilago maydis*, and the level of resistance varies significantly among genotypes. Among the identified genotypes, some exhibited stable and complex resistance traits to various symptoms of the disease.

Based on the analysis of the traits, the genotypes were conditionally divided into three groups:

- High-durability forms
- Medium-durability forms
- Sensitive forms

The levels of both leaf spots and blistering were low in highly resistant genotypes. Although disease symptoms were observed in the intermediate resistant group, the damage did not reach an economically critical level. In the susceptible genotypes, disease development was intensive. These genotypes may be considered promising for use as starting material in breeding programs and may serve as an important genetic resource in the creation of disease-resistant varieties.

At the same time, the collection of traits formed on the basis of the assessments conducted serves as a reliable scientific basis for diagnosing disease resistance in maize, determining selection directions, and improving phytosanitary monitoring.

The fungus *Ustilago maydis*, the causative agent of maize blister blight, is used as a model organism to study dimorphism (the ability to transition into two different forms) in fungi and its role in phytopathogenic development [6], [12]. This pathogen has two main developmental forms: a saprophytic yeast-like phase and a pathogenic filamentous phase [6].

The dimorphic transition process in *U. maydis* involves complex mechanisms such as signal perception, mating, and cellular program reprogramming. In recent years, improvements in reference genomes, high-throughput sequencing, and advances in molecular genetics have further expanded research in this area. However, the biology of other species that are not model organisms is often overlooked, creating uncertainty about the extent to which what is known about *U. maydis* can be applied to other dimorphic fungi.

Our studies suggest that lipids or hydrophobicity may be a common signal that stimulates dimorphic transition in plant-associated dimorphic fungi. However, genomic data alone are not sufficient to fully explain dimorphism among different fungal species.

In general, PCR technology is an important tool for the rapid identification of gene loci associated with resistance and is very effective for determining genetic diversity among maize genotypes. Currently, PCR technology is widely used in the study of plant genomes; the creation of genetic maps, the analysis of the genetic structure of populations, genotyping, marker identification of traits, the analysis of 64 levels of introgression of somatic hybrids, as well as the implementation of all breeding programs. PCR technology is a tool that allows for rapid differentiation of all effects when identifying resistance-related markers in grains, as well as genetic variations between maize genotypes [11]. Marker-based selection significantly accelerates the process of grain production multiplication (breeding) [10].

To achieve maximum effect in maize breeding, it is important to use the right combinations of resistance genes. The effect of gene combinations is not always better than the individual effect. Genes operate in different resistance mechanisms or at different optimal temperatures, which allows them to provide resistance under different conditions. It would be ideal if several pathotype-specific genes were combined with several pathotype-nonspecific APRs. This is because sexual hybridization and other processes in the pathogen produce virulent biotypes and forms that can overcome existing resistance. Therefore, the constant search for such new genes is inevitable [3].

#### **4. Conclusion**

The results of the present study indicate that the response of maize genotypes to common smut disease is directly related to their genetic characteristics [4]. The observed variation in resistance levels suggests that this trait is not controlled by a single gene but rather by complex genetic mechanisms. Identification of highly resistant genotypes provides valuable material for use in breeding programs.

Furthermore, the results demonstrate that disease development depends not only on pathogen virulence but also on the morphophysiological condition of the plant. Genotypes exhibiting vigorous vegetative growth generally showed lower infection levels, which may be explained by their overall physiological resilience. Thus, resistance is determined not only by the presence of specific resistance genes but also by the plant's adaptive capacity.

The application of molecular screening methods enabled the identification of hidden genetic differences among genotypes that appear phenotypically similar. This highlights the importance of marker-assisted selection, as visual observations alone may not provide sufficiently precise information for breeding purposes. The obtained results are consistent with findings from international studies and indicate that increasing genetic resistance represents the most effective strategy for controlling *Ustilago maydis* [3], [4]. Although chemical control may provide short-term effects, the development of resistant cultivars remains a more efficient and environmentally sustainable long-term solution.

Thus, the present study has practical significance for the conservation and enrichment of the maize gene pool and provides a scientific basis for future breeding research.

### Author Contributions

Surayya T. Ismayilova conducted the literature search and drafted the manuscript. Assoc. Prof. Saltanat A. Aghayeva supervised the study, provided critical guidance on content and structure, and reviewed the manuscript.

### Conflict of Interest

The authors declare no conflicts of interest.

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Review Article

# Investigation of the Genetic Basis of Wheat Resistance to Salt Stress

Maryam E. Eyyubova  

Department of Natural Sciences, School of Advanced Technologies and Innovation Engineering, Western Caspian University, 17 A, Ahmad Rajabli Street, III Parallel, AZ1072 Baku, Azerbaijan

## Abstract

This article explores the genetic factors and mechanisms underlying wheat's resistance to salt stress. One of the key strategic food crops that significantly contributes to the security of the world's food supply is wheat. Abiotic stressors, including salt, elevated temperatures, and drought, create an adverse environment that reduces wheat yield and production capacity. In any stage of plant development, salt stress may result in irreversible losses of yield potential in a variety of agricultural crops, such as wheat, barley, and rice. Salinity stress induces osmotic and ionic stresses, which lead to the transfer of considerable amounts of  $\text{Na}^+$  ions into plant cells and an increase in  $\text{Na}^+$  concentration in the cytoplasm and vacuoles. This process can eventually lead to cell death and disrupt metabolic processes. Soil salinity significantly limits wheat production by reducing plant water absorption and inducing nutritional deficits due to ion toxicity, leading to diminished plant growth and yield. Considering the essential role of wheat in global food security, investigating salt tolerance in wheat and creating salt-tolerant breeds is crucial for maximising the use of saline-alkaline soils and guaranteeing sustained food production.

**Keywords:** wheat, salt stress, salt tolerance, genetic mechanisms, abiotic stress, ion toxicity

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## 1. Introduction

Global crop production is in danger because of soil salinity, a crucial abiotic stress factor that lowers agricultural productivity. Climate change, geological and hydrological circumstances, and human activity, particularly excessive irrigation, are the primary drivers causing soil salinity. Consequently, the management of salinization becomes more challenging, and the extent of saline land areas may continue to grow [1]. Wheat (*Triticum aestivum*) is a necessary agricultural crop that is cultivated worldwide and is an essential food in numerous countries. Nevertheless, it is difficult to achieve this demand due to the limited availability of agricultural land and unfavorable climatic conditions. Research indicates that an economic yield, spike indicators, grain mass, and seed number decrease significantly with each unit increase in salinity in both salt-sensitive and salt-tolerant wheat varieties. Excessive accumulation of primarily  $\text{Na}^+$  and  $\text{K}^+$  ions in plant cells is the outcome of soil salinization, which eventually culminates in severe ion toxicity. Furthermore, secondary stress factors like oxidative damage and osmotic stress are produced by salt stress and can interfere with metabolic activities. This review summarizes recent research on the molecular regulatory mechanisms of salt tolerance in wheat and aims to form a theoretical basis for improving breeding efforts by discussing the potential of new technologies to increase salt tolerance in wheat. The high toxicity of salt ions in the soil disrupts the formation of photosynthetic pigments due to the inhibition of the biosynthesis of various chlorophyll components. In wheat, chlorophyll content (CC), relative water content (RWC), normalized difference vegetation index (NDVI), and membrane stability index (MSI) are significantly reduced under the influence of soil salinity.

As salinity levels increase, the amount of water reaching the roots decreases, resulting in poorer nutrient uptake by wheat plants through the root system. In response to salinity stress, changes occur in key physiological functions, including protein synthesis, energy production, and lipid metabolism, during the embryonic stages of the plant. As a result of the drastic weakening of plant growth, the synthesis of toxic metabolites and molecular damage can occur, which ultimately leads to the destruction of the plant [2]. To combat stress, plants re-regulate their metabolism, both enzymatic and non-enzymatic antioxidant mechanisms.

In order to mitigate the osmotic stress induced by salinity, plants synthesise and accumulate metabolically compatible solutes, including proline, carbohydrates, and betaines. Proline functions as an osmoprotectant, assisting plants in maintaining a tissue water potential that is lower than that of the soil. This process, in the end, maintains the turgor pressure that is essential for growth [3]. In addition to maintaining osmotic balance, these compatible solutes also have an antioxidant function, helping to stabilize subcellular structures and regulate intracellular redox potential under stress conditions.

Salinity stress impacts the quantitative feature of wheat grain yield. Salinity stress impairs the growth and development of spikelets during the reproductive and maturation stages. Salinity stress has a detrimental impact on the height, spike length, and grain weight of plants. The production of specific bread products from wheat is directly related to grain quality. This property is evaluated based on physical and compositional parameters such as grain hardness (GH), protein content (PC), and moisture content (MC). The effect of salt stress on wheat quality indicators is a relatively understudied area. Therefore, a two-year study was proposed to evaluate the effects of salinity stress on physiological, biochemical, yield, and other quality-related parameters in 20 wheat genotypes of diverse genetic origin, in order to identify parameters that can be used as biomarkers for rapid selection and improvement of salinity-tolerant varieties. In this study, it was hypothesized that the response of wheat quality to salinity stress would differ between genotypes.

## **2. Effects of Salt on Wheat and Its Physiological Basis**

The main stress factors that salt causes in plants are mainly related to ionic toxicity and osmotic stress. The growth of plants is directly influenced by osmotic stress, which restricts the expansion of root ends and leaf cells. The effects of ionic stress on plant growth usually become apparent at later stages. In plants with the ability to transport  $\text{Na}^+$ , the effects of ionic stress are often not considered as critical as osmotic stress. The effect of salt stress on aboveground plant growth is observed in two phases: first, a rapid response to an increase in external osmotic pressure, and then a slow response phase to  $\text{Na}^+$  accumulation in the leaves. In the first stage, the development of new shoots is weakened, and in the second stage, an accelerated senescence process occurs in older leaves. In particular, in cereal plants, a significant decrease in bushiness and spike number is observed under the influence of salt stress in the initial stage. A decrease in cellular water potential as a result of osmotic stress can limit water use in plants. When exposed to salt stress, a decrease in relative water content (RWC) is observed in both salt-tolerant and salt-sensitive varieties, with a weaker expression in salt-tolerant wheat varieties [4]. Salt stress also stimulates the formation of reactive oxygen species (ROS) such as superoxide anion ( $\text{O}_2^-$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), and hydroxyl radical (OH). Excessive accumulation of ROS creates oxidative stress, which negatively affects plant growth and development by causing cellular toxicity through damage to membrane lipids and oxidation of proteins [5]. The main source of ROS in plants is NADPH oxidases (NOXs), also known as respiratory burst oxidase (Rboh).

## **3. Strategies to Improve Salt Tolerance of Wheat**

### **3.1. Breeding**

Hybridization, a classic method in plant breeding, has played a significant role in shaping desired characteristics in wheat. The complicated and polygenic trait of salt stress tolerance is regulated by several genes. The selection of salt-tolerant wheat varieties is significantly influenced by a comprehension of the genetic basis of salt tolerance. In order to reduce the adverse effects of salt stress, plants that are tolerant of it apply a range of morphological, physiological, and biochemical strategies, such as ion excretion, the formation of organic osmolytes, the synthesis

of antioxidants, and modifications in nutrition. There are about 350 species in the Triticeae tribe, which includes wheat's wild relatives. There is an enormous amount of genetic variability among its members, in particular when it is related to salt tolerance. Species belonging to the genera *Elytrigia* and *Thinopyrum* are mainly distributed in coastal areas, while *Leymus* species thrive in soils with high salinity and alkalinity. Long wheatgrass (*Thinopyrum ponticum*), a halophyte relative of wheat, is considered one of the plants with the highest salt tolerance and can survive even in conditions of salinity close to seawater. The differences in salt tolerance observed among these species provide favorable opportunities for increasing the salt tolerance of wheat through hybridization and indicate that wild species are a potential source for improving wheat's resistance to salinity. Unlike disease resistance, salt tolerance in wheat is considered more difficult to assess in the breeding process because it is a complex trait controlled by several genes. Since the 1980s, interspecific hybridization techniques have been used to transfer salt-tolerant genes from Triticeae species to common wheat. However, despite the passage of about 25 years since the initial experiments, the new varieties obtained have not yet been widely adopted by farmers due to difficulties related to productivity and ecological adaptation.

### **3.2. New Breeding Techniques**

Although traditional hybridization has achieved significant success, several challenges remain, including the relatively low use of plant chromosomal translocation lines in wheat breeding programs. Furthermore, the integration of desirable traits such as salt tolerance is often limited due to problems such as incompatibility and hybrid sterility in distant hybridization. In order to resolve these challenges, it is necessary to incorporate present molecular biology methodologies, including gene editing, transgenic technologies, and molecular marker-assisted selection, into wheat breeding. These approaches can increase selection efficiency and ensure precise regulation of target traits.

Molecular marker-assisted selection combines traditional genetic selection with modern molecular biology methods to produce plant materials with superior genetic properties. This approach, known as marker-assisted selection (MAS), is widely used worldwide to develop various traits in wheat, particularly to improve key economic traits. Several Turkish genotypes and Australian salt-tolerant genotypes were chosen as repeat parents using the MAS approach on the *Nax1* and *Nax2* genes, and four backcrosses were carried out to generate new wheat genetic material. Generation combinations containing salt tolerance genes were obtained as a consequence of this methodology [6].

MAS enables the combination of tolerance components from various genetic sources, facilitates multiple selection in a relatively short time, and allows for early selection of target traits, as salt tolerance in plants typically involves multiple traits and mechanisms. This approach can be considered a significant tool for improving the salinity tolerance of plants. Compared to traditional field evaluation and phenotypic selection, MAS offers a promising approach for the creation of plants with increased salt tolerance. Although numerous QTLs associated with salt tolerance have been identified in various plant species, the development of commercial varieties or breeding lines with improved salt tolerance through MAS remains limited [7].

Due to the complex nature of soil salinity and the difficulties in distinguishing its effects on infiltration processes, it has been difficult to identify reliable loci for salt tolerance and related traits. However, with the gradual increase in data, especially on the wheat genome, QTL mapping has emerged as a crucial tool for uncovering the genetic basis of complex plant traits. The relationship between chlorophyll content and overall biomass characteristics and some physiological parameters suggests that the  $\text{Na}^+$  and  $\text{K}^+$  accumulation pathways are genetically distinct. Numerous molecular markers linked to genes or QTLs affecting necessary traits have been identified and proposed as potential indirect selection criteria to improve selection efficiency through MAS. Identification of quantitative trait loci (QTLs) remains an effective and reliable approach for studying the genetic basis of salt stress resistance in wheat. QTL mapping allows for the precise localization of genomic regions responsible for traits associated with stress resistance. Research conducted from 1996 to 2022 identified 934 significant quantitative trait loci (QTL) linked to salt stress resistance in wheat. These QTLs covered 21 germination traits, 23 agronomic traits, and 12 ionic traits and were confirmed with  $\text{LOD} \geq 3$  in 26 studies. Large-scale genome-wide studies have identified over 500 QTLs associated with salt stress response and have shown that each QTL explains between

8.4% and 40.0% of the phenotypic variation. In a study investigating the genetic basis of wheat seed germination and traits under salt stress conditions, 31 unconditional QTL and 11 conditional QTL associated with 16 candidate genes related to salt stress resistance were identified. Genome-wide association studies (GWAS) have identified significant QTLs associated with chlorophyll content (CC) and specific leaf area (SLA) under salt stress conditions on chromosomes 1B, 2B, 2D, 3A, 3B, 5A, 5B, and 7B. Functional annotation of genes revealed three key candidate genes - TraesCS5A02G355900, TraesCS1B02G479100, and TraesCS2D02G509500, whose expression was significantly increased under salt stress conditions [8].

### 3.3. Transgenic Technology

Transgenic Technology acts as a crucial optimization strategy in wheat breeding and allows for increased disease resistance, stress tolerance, and yield through the introduction of foreign genes. This technology plays a significant role in creating wheat plants that aim to increase productivity and quality indicators in response to changing environmental conditions and human needs.

### 3.4. Exogenous Application

The results are occasionally uncertain, and the selection process is time-consuming. Conversely, the most direct method of enhancing salt tolerance in wheat is the exogenous application of specific plant growth regulatory compounds [9]. Examples of these substances include osmoprotectants, signalling molecules, antioxidants, and plant nutrients. Interaction with these foreign compounds leads to the creation of a specific physiological state in the plant, in which various defense mechanisms are activated. Crops are capable of responding to salt stress immediately and effectively as a consequence of this activation. Osmoregulation is a critical adaptive mechanism that enables plants to sustain turgor pressure in stressful environments and enhances their tolerance to drought or salinity at the physiological level. The significant function of osmotic agents is supported by plenty of data from a variety of in vitro and in vivo studies that utilise physiological, biochemical, genetic, and molecular methodologies. These substances, which are involved in enhancing the resistance of plants to both drought and salt stress, include sugars and sugar alcohols (trehalose, D-ononitol, sorbitol, fructose, and mannitol), amino acids (such as proline and exobases), and ammonium compounds (O-choline sulphate,  $\beta$ -alanine betaine, polyamines, glycine betaine, and dimethyl sulfopropionate).

Crop growth necessitates optimal plant nutrition. Nutrients support plant growth and functionality, and they additionally assist wheat crops in managing the adverse effects of salt stress. Excessive salinity causes nutritional lacks that interfere with normal plant growth and metabolism by increasing the absorption of  $\text{Cl}^-$  and  $\text{Na}^+$  while decreasing the absorption of  $\text{Zn}^{2+}$ ,  $\text{Ca}^{2+}$ , and  $\text{K}^+$ . K, N, phosphorus (P), magnesium (Mg), silicon (Si), selenium (Se), and zinc (Zn) are the crucial nutrients for plants. By stimulating respiratory enzymes, boosting photosynthetic efficiency, and supporting osmotic management, potassium is known to improve wheat's resistance to salt. For regulating the flow and distribution of  $\text{Na}^+$ ,  $\text{Cl}^-$ , and mineral ions, external Si application improves wheat growth under salt stress. It is believed to be an effective method of increasing crop tolerance to salt in the field [10]. Additionally, by lowering reactive oxygen species (ROS) under salt stress, Se application may reduce wheat damage.

### 3.5. Non-Enzymatic Antioxidants and Exogenous Signaling Molecules

Ascorbic acid (AsA), glutathione (GSH), tocopherol, and other non-enzymatic antioxidants are crucial in suppressing ROS levels in plants. Salt stress significantly increases lipid peroxidation, leading to a marked increase in the activity of antioxidant enzymes in plants [11]. Application of AsA to wheat roots increases endogenous AsA levels and catalase (CAT) activity, helping to maintain ion homeostasis and improve photosynthetic potential, thereby reducing the negative effects of salt stress on some wheat varieties.

Correlation analysis and principal component analysis (PCA) were performed to understand the variability in adaptation mechanisms in wheat plants under control and salt stress conditions. The limited success in selecting salinity-tolerant wheat varieties can be attributed to the absence of precise indicators for physiological and

agronomic traits associated with salinity stress, as well as the low genetic diversity present in current wheat genetic resources. Although varieties previously described as salt-tolerant have been used in breeding efforts, the results obtained have been limited due to a lack of understanding of the mechanisms of salt tolerance. Most of the areas prone to saline soils are currently unused. Cultivation of salt-tolerant wheat varieties on saline soils can be of enormous importance in terms of attracting these areas to agriculture and meeting the food needs of the ever-growing population. This will be possible by clarifying the mechanisms behind differences in salt tolerance between different wheat varieties and identifying salt-tolerant genotypes.

#### **4. Conclusion**

There are still several necessary questions waiting to be discovered. In addition to the known and putative ion receptors, are there salt receptors in the plasma membrane, cytoplasm, and organelles of plant cells? How do responses to salt stress interact with other environmental stresses? How can plant growth and productivity be linked in salt-affected areas? The degree of damage caused by salt to plants depends on many different factors: the growth stage of the plant, ion levels, genotype, temperature, plant organs exposed to salt, the composition of the saline solution, and the duration of salt exposure. As a result of continuous developments in scientific and technological methods, the genetic and molecular basis of salt tolerance in wheat is increasingly being elucidated.

Wheat's tolerance to salt stress can be greatly increased by the development of novel salinity-tolerant varieties and genetic advancements, and notable progress has been made in this area. A better comprehension of the complex characteristics associated with salinity in wheat has been made possible by the use of current technologies and ongoing advancements in this field. Simultaneously, there is a growing interest in the breeding of wild relatives of wheat and the transfer of beneficial genes from them. Nevertheless, this particular area continues to confront numerous obstacles. Initially, there are several limitations on the genetic transformation of wheat, including a small selection of wheat varieties that can be transformed and a poor transformation efficiency. These limitations prevent the efficient and stable transfer of essential salt stress resistance genes to target varieties. In order to address these challenges, researchers have created growth regulatory factors (GRFs) and their cofactor-interacting factors (GIFs) to improve the regeneration and transformation capabilities of plants.

#### **Author Contributions**

The author prepared the concept and methodology, reviewed, and edited the article.

#### **Conflict of Interest**

The author declares no conflicts of interest.

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Review Article

# Initial Diagnosis of Barley Rust Disease Based on Visual and Morphological Signs

Saida N. Burjaliyeva ✉ 

Department of Natural Sciences, School of Advanced Technologies and Innovation Engineering, Western Caspian University, 17 A, Ahmad Rajabli Street, III Parallel, AZ1072 Baku, Azerbaijan

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## Abstract

Barley is among the widely distributed and commercially necessary cereals in the world. However, diseases triggered by different kinds of plant pathogens pose a serious challenge to barley production. Some of these diseases are the rusts that are caused by various fungi belonging to the Puccinia species. The main characteristic of the rust disease in barley is its wide distribution and the fact that it leads to crop losses. Early detection of the rust disease is important for its management and containment. The presented article examines the possibilities of initial diagnosis of rust disease in barley plants based on visual and morphological indicators. The morphological characteristics of the pathogen, the color, shape, and location of the blotches, and the symptoms observed on the leaf surface of barley samples infected with rust disease were analysed during the study. The findings suggest that visual and morphological indicators can be employed as an effective and practical method for the early detection of rust disease in initial diagnostics. This method enables the implementation of operational decisions in the field, thereby reducing crop losses.

**Keywords:** barley, rust disease, uredospores, initial diagnostics

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## 1. Introduction

Barley is one of the most important grain crops cultivated by humans since ancient times (*Hordeum vulgare* L.). Nearly 9,000 years ago, barley was first cultivated in the Middle East and has since become a globally significant food and livestock agricultural crop. Barley's capacity to endure severe climatic conditions enables it to cultivate grains in both arid and cold regions. As a result, barley is the fourth most abundant crop in the world in terms of both production volume and cultivated area, following rice, maize, and wheat. In addition to its culinary applications, barley is essential for both animal husbandry and industry [1].

A number of significant factors are indicative of the agricultural importance of barley. Barley is initially highly adaptable to a wide range of soil types and climates. It is a dependable crop for global cultivation due to its ability to survive in both semi-arid and cold environments. Secondly, barley's short growing season results in a higher yield than other cereals. Barley's contribution to global food security is demonstrated by the fact that it is grown on millions of hectares across the world and yields hundreds of thousands of tonnes of crops annually.

There are numerous nutritional and commercial uses for barley. Animal feed, particularly for dairy animals like cows and sheep, uses a significant amount of the entire crop. It also provides raw materials for the brewing industry. In some regions, it is also used to a significant extent for human consumption [2]. Barley is high in protein, fiber, and vitamins and minerals, which increases its value both in terms of food and industry. These properties are also crucial in protecting animal health.

In terms of productivity, barley is determined by various factors. Genetic characteristics, cultivation conditions, soil type, and wet and dry environments directly affect yield indicators. Plant height, number of ears, number of grains per ear and 1000-grain weight of barley varieties are evaluated as the main components of productivity. For

example, varieties with dense heads and large grains have higher yield potential. In addition, soil and irrigation conditions also affect yield. Yields can be 20-30% higher in well-irrigated areas [3]. Modern agricultural research evaluates the agro-morphological characteristics of different genotypes to increase barley productivity. As a result of these studies, the identification of high-yielding varieties and their application in breeding programs creates conditions for a continuous increase in productivity. The scientific works prove the direct relation between the biomass and the vegetation index of some barley genotypes and productivity. This means that choosing the right genotypes plays an important role. In addition, one should consider that barley is an indispensable agricultural product in Azerbaijan. It is the main food resource for cattle and grows everywhere in Azerbaijan. Some varieties of barley are grown in saline and low-fertility soils. Thus, the adaptability to many types of ecology has been proven by the studies. Modern breeding methods enable people to preserve and use the genetic resources of barley effectively in order to ensure productivity. The main purpose of developing barley genotypes is to raise productivity and secure food safety. Healthy soils and good cultivation practices are not the only reasons why this agricultural product is so productive. The good condition of plants plays an equally important role as well. Diseases affecting the leaves, stems, and grains may affect the plant negatively. They can influence photosynthesis and nutrient distribution. For this reason, it is necessary to establish appropriate management strategies and detect illnesses among barley fields at the right time in order to ensure sustainability in yield.

One of the many illnesses affecting agricultural areas is called barley rust (*Puccinia hordei* or other types of *Puccinia*). This disease leads to huge losses and is typical for the natural environment as well as for agricultural lands. Spots on the leaves of barley plants look like rust color and are connected with yellow and brown stains on leaves caused by this disease [4]. Such signs lead to a decrease in photosynthesis, energy accumulation, and grain growth. In conclusion, ensuring the productivity of barley plants involves managing diseases, as well as soil and fertilizing conditions. It means that specialists should conduct thorough research about symptoms, ways of infection spread, and prevention of this disease in order to avoid crop losses.

As it was already noted, the sustainability of agrarian significance depends on the capacity of growing this type of crop productively. Nevertheless, various stressors related to plant biology can limit the capacity of growing barley. Among these diseases is a rust, a condition that affects physiological processes within the plant while it is in the vegetative stage and leads to low productivity. Analyzing the biological and physical properties of the pathogen scientifically, as well as symptoms and the mechanisms of transmitting the disease, it is possible to better understand what triggers the decrease in productivity. At the same time, identifying rust disease accurately will be extremely useful for designing ways to overcome this problem.

Barley (*Hordeum vulgare* L.) rust is mainly caused by fungal pathogens and is considered one of the most widespread phytopathological problems in barley production worldwide, causing yield reductions. The causative agents of rust disease are obligate parasitic fungi belonging to the Basidiomycota division, the Pucciniales order, and mainly the *Puccinia* genus. Since these pathogens can only grow in living plant tissue, they have the ability to spread rapidly and cause widespread infection in the field. The main fungal species that cause rust in barley differ somewhat in the symptoms they cause in the plant and the form of infection. The most common rust species include leaf rust, yellow rust, and, to a lesser extent, stem rust [5].

## **2. Leaf Rust Pathogen (*Puccinia hordei* G.H. Otth)**

The main cause of barley leaf rust is the fungus *Puccinia hordei*. This pathogen develops mainly on the leaf surface and manifests itself in the relatively late stages of the growing season. After entering the leaf tissue, the fungus develops under the epidermis and produces uredospores. As a result, round or oval brown or orange rust pustules form on the leaves. *P. hordei* develops rapidly in conditions of high humidity and mild temperatures. The disease weakens the photosynthesis process and causes premature drying of the leaves, which leads to serious yield losses during the grain-filling stage. The physiological deterioration of the barley plant is typically observed alongside leaf rust, which can be clearly identified visually in field conditions.

### **3. Yellow Rust Pathogen (*Puccinia Striiformis* Westend f. sp. *hordei*)**

*Puccinia striiformis* f. sp. *hordei* is suspected to be the cause of yellow rust disease, a pathogen that is particularly hazardous in humid and cold climates. The primary visual indicator of yellow rust is the presence of yellowish pustules that are arranged in parallel stripes on the leaf surface. This is one of the primary signs that differentiate yellow rust from other rust varieties. This pathogen has a more serious negative impact on barley plant development because it can become active in the early vegetative stage. In plants infected with yellow rust, a large part of the leaf surface loses its functional activity, resulting in a weakening of the overall development of the plant and a sharp decrease in yield. Since *P. striiformis* is highly adaptable, the emergence of new physiological races is also possible, which makes disease control difficult.

### **4. Stem Rust Pathogen (*Puccinia graminis* Pers f. sp. *tritici* Erikss. & Henning)**

Although less common in barley, stem rust is also a potentially dangerous disease. The causative agent of this disease, *Puccinia graminis* f. sp. *tritici*, is mainly characteristic of wheat, but under certain conditions, it can also infect barley plants. Stem rust is observed as the formation of elongated, dark-colored rust spots on the plant stem, leaf sheaths, and sometimes the spikes. This pathogen weakens the mechanical resistance of the plant, causing stem breakage and plant dormancy. As a result, crop losses increase not only in terms of grain quality but also in terms of difficulties during harvesting.

### **5. General Biological Characteristics of Rust Pathogens**

One common characteristic of the fungi that cause barley rust is that they have a complex development cycle. These pathogens produce different types of spores (uredospores, teliospores, and basidiospores) during their life cycles. In particular, uredospores play a key role in the rapid spread of the disease during the growing season. Rust has the potential to rapidly infect huge areas of cropland due to its ability to be transported over great distances by the wind.

### **6. Optimal Conditions for the Evolution of Rust Disease**

The environmental factors and agrotechnical conditions are directly correlated with the spread and intensive development of rust disease in barley. Rust pathogens are obligate parasitic organisms, which means that their development is dependent upon the presence of susceptible plants and the presence of appropriate environmental conditions. Accordingly, the emergence and rapid spreading of rust disease frequently occur as a consequence of the simultaneous action of numerous factors [6].

### **7. Meteorological and Climatic Parameters**

The optimal conditions required for the proliferation of rust are when there is moisture-laden air along with mild temperatures. This means dew drops formed on leaves, particularly after heavy rains or during the night period, form an excellent environment for the growth of fungal spores. For the germination of uredospores and their entry into the plant's tissue, the leaf must remain moist for some period. Moreover, rust disease also depends on temperature levels. In general, rust fungi operate within the range of 15 to 25 degrees Centigrade. Though yellow rust grows well in low temperatures, leaf rust might adjust well in warm conditions [7]. The ideal mix of humidity and warmth allows the disease to spread quickly over wide areas.

### **8. Physiological and Developmental Stage of the Plant**

Barley plants' vulnerability to rust disease varies during the growing season. Between the tillering and heading periods, the disease is at its most active. Pathogens can more easily enter plant tissue during this time due to the broad leaf surface and high photosynthetic intensity. Rust is more likely to affect plants that are undernourished, agitated, or damaged by other diseases. The application of nitrogen fertilizers in excess results in an increase in vegetative growth, which in turn creates a favorable environment for rust pathogens by increasing the humidity of the microclimate.

## **9. Agricultural Technical Parameters**

The proper or improper implementation of agrotechnical measures significantly influences the spread of rust disease. Densely planted areas weaken air circulation, and prolonged moisture on the leaf surface accelerates the development of fungal infections. At the same time, keeping diseased plant residues in the field allows pathogens to continue their activity in the next growing season. Long-term barley cultivation as a monoculture leads to an increase in sources of infection in the soil and the environment. Failure to apply a crop rotation system leads to the recurrence of rust disease every year and its manifestation at earlier stages.

## **10. Variety Characteristics and Genetic Susceptibility**

The level of genetic resistance of barley varieties to rust disease directly affects the rate of disease development. Susceptible varieties can be massively infected in a short period of time under suitable climatic conditions. Resistant varieties, on the other hand, limit the development of pathogens to some extent, thereby slowing down the spread of the disease. However, the high variability of rust pathogens can infect even previously resistant varieties in some cases. This factor is one of the main reasons why rust disease remains a persistent phytosanitary problem.

## **11. External Sources of Infection and Routes of Spread**

One of the significant factors affecting the development of rust disease is the mechanism of infection spread. Rust spores can be carried very long distances by the wind. Therefore, the disease can be transmitted not only within a field but also from neighboring crops. When suitable climatic conditions are created on a regional scale, rust disease can infect large areas in a short time [8]. As a result of the influence of the mentioned ecological and agrotechnical factors, rust disease is accompanied by characteristic visual and morphological changes in barley plants, which allow for initial diagnostics in field conditions.

## **12. Initial Diagnosis Based on Visual Signs of Rust Disease**

Visual observations play a key role in the initial diagnosis of rust disease in the field. The characteristic external signs produced by the pathogens of the disease allow us to determine the presence of infection even without laboratory analysis. Correct assessment of visual symptoms is necessary in differentiating rust from other leaf spot diseases and in taking timely control measures.

## **13. Symptoms Observed on Leaves**

The initial visual symptoms of rust disease are most often observed on the leaves of barley plants. In the early stages of the illness, the leaf surface develops tiny, yellowish or light orange dots. As they age, these patches enlarge and turn into brown, orange-brown, or yellow pustules. The pustules typically burst through the leaf tissue, emerging from the leaf epidermis. On the leaf surface, rust pustules can appear irregularly or in a specific pattern. Leaf rust spots are typically observed in dispersed patterns, while yellow rust pustules form parallel bands along the leaf veins. Usually, the pustules emerge from the leaf epidermis after rupturing through the leaf tissue. Rust pustules can show up randomly or in a particular pattern on the leaf surface. While yellow rust pustules form parallel bands along the leaf veins, leaf rust patches are usually found in scattered patterns [9]. This characteristic is thought to be essential for identifying different forms of rust in the field. As the disease progresses, the number of pustules increases, merging to cover a large part of the leaf surface. In the end, the photosynthesis process weakens, the leaves turn yellow prematurely, and they start to dry out. Severe infections sharply reduce the functional activity of the leaves, negatively impacting the overall development of the plant.

## **14. Signs on the Stem and Leaf Sheath**

In the advanced stages of rust disease, the barley plant's stem and leaf sheaths also exhibit visual symptoms. These symptoms are especially pronounced during stem rust. Oblong, dark brown or reddish-brown rust spots appear on the surface of the stem and leaf sheaths. The appearance of these areas adversely affects the mechanical strength

of the tissue of the stem and leads to its epidermis cracking. Thus, the plant is more susceptible to the impact of outside factors. In cases of acute infection, the weakness in the stem leads to the falling of the plant falling to the ground; it is prone to lying on the soil surface. The transmission of nutrients by the plant is even more restricted because of rust indications on the leaf sheaths.

### **15. General Morphological State of the Plant**

In the case of visual identification of rust disease, the overall morphology of the plant and the signs on the organs under observation are regarded as important indicators. Plants affected by rust disease possess low vegetative biomass compared to healthy plants. Barley plants exhibit early signs of ageing with severe infestations. Early leaf drying prevents the plant from properly absorbing nutrients. During the grain filling stage, this situation is very noticeable [10]. Because of this, the grains that form in the spikes are weak, tiny, and light in weight. In cases where the rust disease persists for a long time, the plants become sparse, and uneven development is observed in the field. These morphological changes are considered one of the main indicators that allow assessing the intensity of the disease and conducting initial diagnostics in field conditions.

### **16. Diagnostic Indicators Based on Morphological Features**

In addition to visual observations, the study of the morphological characteristics of the pathogen is of particular importance in the accurate diagnosis of rust disease. Morphological diagnosis is mainly based on the examination of fungal spores under a microscope, and this method allows the differentiation of rust disease from other leaf spot or necrotic diseases. Although symptoms observed in the field provide preliminary information, morphological indicators play a significant role in determining the causative agent of the disease [11].

### **17. Morphological Characteristics of Uredospores**

The most common spore type during the active spread of rust disease is uredospores. These spores are mainly formed within rust pustules that form on the leaf surface and play a key role in the spread of the disease during the growing season. Uredospores are usually oval, elliptical, or ovoid in shape under the microscope. Their color is yellow-brown or orange-brown. The surface of the uredospores is covered with small spiny protrusions, which is one of the characteristic morphological features of rust fungi. The relatively thin but flexible spore wall allows them to be easily dispersed by wind. These morphological features allow uredospores to be distinguished from spores of other phytopathogenic fungi.

### **18. Morphological Characteristics of Teliospores**

In later stages of the disease, teliospores are formed in the rust pustules. Teliospores are thicker-walled and more durable than uredospores. These spores are usually dark brown or black in color and are characterized by their two-celled structure. The main function of teliospores is to ensure the survival of the pathogen in unfavorable conditions. Microscopic observation reveals that the tips of teliospores may become thickened, and the distinct visibility of the septum between cells enhances their diagnostic value. These features allow us to determine the stage of development of the rust disease and assess its phenological period.

### **19. Color and Location of Spore Mass**

In morphological diagnosis, both the morphology of the spores and their colors, and even the mannerisms in which the spores appear on plants, are considered important criteria. Orange-brown in color and scattered on the leaves, spore masses of leaf rust appear. In yellow rust, spore masses appear in stripes on the leaf veins with a pale-yellow color. Spore masses of stem rust have dark colors, and long pustules can be observed on the leaves and sheaths of the stem. Morphological characteristics become highly valuable in distinguishing among rust species [12].

## 20. Additional Microscopic Diagnostic Parameters

The dimensions, wall thickness, and texture of the spores, along with the condition of the host tissue, are considered while making a diagnosis of rust pathogens. In the affected tissues, there is growth of hyphae between the cells and destruction of epidermal cells. This indicates that the parasite is parasitizing the plant tissues. When the two methods are used together, the accuracy of the diagnosis improves immensely. The rust disease can be accurately diagnosed, particularly when laboratory tests confirm the results found initially in the field.

## 21. Conclusion

According to the findings of the analysis, rust disease is a severe phytopathological problem that affects barley's (*Hordeum vulgare* L.) grain yield and quality. The disease-causing fungus of the genus *Puccinia* is extremely adaptive and can spread quickly in favorable ecological and agrotechnical settings. This element emphasizes how crucial it is to identify rust disease in the environment as soon as possible. The results of the study demonstrate the importance of visual indicators in the early detection of rust illness. By looking for the distinctive rust pustules on the leaves, stems, and leaf sheaths, as well as the development of striped or scattered patches on the leaf surface, the disease can be quickly detected in the field. In the meantime, the overall morphological state of the plant, which includes early yellowing, slow growth, and core shrink is used to determine the severity of the disease.

The study of morphological characteristics, particularly the microscopic characteristics of uredospores and teliospores, alongside visual symptoms, greatly enhances diagnostic precision. The disease can be differentiated from other leaf spot pathogens, and the specific pathogen can be determined based on the particle's shape, color, cell wall construction, and localization [13]. It can be concluded that the early detection of rust disease through visual and morphological symptoms renders its prevention highly important both scientifically and practically. This method reduces crop loss in barley cultivation, and at the same time increases the efficiency of plant disease prevention techniques. In future research, combining molecular techniques with diagnostic methods may help build more efficient control strategies against rust disease.

## Author Contributions

The author conceived and designed the study, conducted the literature review, analyzed the data, and wrote the article. The author reviewed and approved the final version of the paper.

## Conflict of Interest

The author declares no conflicts of interest.

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Editorial Office Address: 17 A, Ahmad Rajabli Street,

III Parallel, Baku, Azerbaijan

Phone: +994 12 565 39 77

Email: [rjaa@wcu.edu.az](mailto:rjaa@wcu.edu.az)

<https://rjaa.wcu.edu.az/>

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