Research Article

Molecular Screening of *Ustilago maydis* Fungus in Maize Genotypes

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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

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 petioles [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 β-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 60^0C 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 40^0C 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 $1.8 \leq [D260/D280] \geq 2$, 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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