MOLECULAR IDENTIFICATION OF MONILIOSIS DISEASE IN LOCAL APPLE (*MALUS DOMESTICA*) CULTIVARS FROM THE GUBA–KHACHMAZ REGION OF AZERBAIJAN

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Abstract: In modern agriculture, the protection of fruit crops from diseases is of particular importance for increasing productivity and improving quality parameters. Apple (*Malus domestica*) is one of the most important fruit crops both in the food industry and in terms of economic value. However, various phytopathogenic fungi, especially those belonging to *Monilinia* spp., which cause brown rot (moniliosis), have a significant negative impact on yield and postharvest quality. Moniliosis is mainly caused by *Monilinia fructigena*, *M. laxa*, and *M. fructicola*, and it leads to substantial economic losses both in the field and during the postharvest period. Disease is characterized by the formation of decay lesions on fruits, tissue degradation, and intensive sporulation. The aim of this study was to comprehensively evaluate the susceptibility and resistance levels of local apple cultivars from the Quba-Khachmaz region of Azerbaijan to *M. fructicola*. For this purpose, the apple cultivars Gandi-Sinab, Cirhaji, Gizil Ahmedi, and Sari-tursh were artificially inoculated, and the progression of the disease was assessed through visual observations. In addition, the growth characteristics of the pathogen on culture media were examined, its morphological features were determined by microscopic analysis, and molecular identification of *M. fructicola* was carried out using real-time PCR. The obtained results are of great importance for evaluating the resistance of local apple cultivars to moniliosis and providing a scientific basis for the selection of resistant varieties in future breeding programs.

Keywords: apple, *Monilinia fructicola*, moniliosis, molecular analysis, real-time PCR.

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Introduction

Apple (*Malus domestica*) is one of the most widely cultivated and economically important fruit crops in the global horticultural system. It is characterized by high productivity, wide adaptability, and the ability to grow under diverse climatic conditions (Petróczy et al. 2012). Apples are considered a major raw material source both for fresh consumption and for the processing industry. A significant portion of global apple production is consumed fresh, while the remaining part is used to produce juice, canned products, dried fruits, and other processed goods. Apple fruits contain 10–15% sugars, organic acids, pectins, phenolic compounds, vitamins (especially vitamin C), and various mineral elements, which enhance their nutritional and health value (Bustin et al. 2010).

The extensive utilization of apples makes the protection of this crop an important agrobiologically issue. The spread of diseases in orchards not only reduces yield but also deteriorates fruit quality and shortens storage

life. Fungal diseases pose serious challenges in apple production and lead to considerable economic losses. Therefore, the study of major pathogens affecting apple, as well as their distribution patterns and mechanisms of action, remains a relevant scientific issue (Vasić et al. 2013).

One of the most widespread and economically significant diseases of apples is moniliosis, also known as brown rot. This disease is caused by *Monilinia fructigena*, *M. laxa*, and *M. fructicola*, and it affects fruits both during the growing season and in the postharvest period (Riccioni & Valente 2015). Moniliosis initially appears as small brown spots on the fruit surface, which gradually expands, leading to tissue softening and decay. At advanced stages, sporulation structures develop on the fruit surface, facilitating rapid pathogen spread (Jia et al. 2024).

Monilinia spp. are necrotrophic pathogens that invade plant tissues and degrade cellular structures to obtain nutrients. These pathogens typically enter through wounds or mechanically damaged tissues, although they can also infect healthy tissues under favorable conditions. High humidity and moderate temperatures (20–25°C) are key factors promoting disease development. The spores are disseminated by wind, rain droplets, and insects, contributing to the formation of new infection sites (Côté et al. 2004). The taxonomic classification of moniliosis pathogens is as follows:

Kingdom: Fungi
Phylum: Ascomycota
Class: Leotiomycetes
Order: Helotiales
Family: Sclerotiniaceae
Genus: *Monilinia*
Species: *Monilinia fructigena*, *M. laxa*, *M. fructicola*

The severity of the disease depends on several factors, including climatic conditions, fruit maturity stage, and cultivar characteristics (Poniatowska et al. 2021). Infection levels are generally higher under high humidity and during the ripening stage of the fruit. Fruit skin thickness, chemical composition, and inherent defense mechanisms also play an important role in resistance. Soft-textured and sweet fruits are usually more susceptible to infection, whereas fruits with higher acidity and firmer texture tend to exhibit relatively higher resistance (Hrustic et al. 2012).

Although chemical control methods are widely used against moniliosis, they are costly and pose environmental risks (Van Brouwershaven et al. 2010). Moreover, the development of fungicide resistance in pathogens reduces the effectiveness of these treatments. Therefore, enhancing genetic resistance and selecting resistant cultivars is considered a more sustainable and efficient approach (Chen et al. 2013).

In recent years, molecular techniques, particularly real-time PCR, have been widely applied in plant disease diagnostics. This method allows accurate and early detection of pathogens. The cycle threshold (Ct) value provides indirect information about pathogen quantity, enabling comparative evaluation of susceptibility levels among different cultivars (Jia et al. 2024).

The Quba-Khachmaz region of Azerbaijan is an important area for apple cultivation, where several local apple cultivars are widely grown. Investigating the resistance of these cultivars to diseases, particularly moniliosis, is of great importance for preserving local genetic resources and supporting breeding programs (Chen et al. 2013).

The main objective of this study was to perform molecular identification of *Monilinia fructicola* in local apple cultivars from the Quba-Khachmaz region and to comprehensively evaluate their resistance to moniliosis. The study involved artificial inoculation, pathogen cultivation on nutrient media, microscopic analysis, and real-time PCR, allowing a comparative assessment of different cultivars (Boehm et al. 2001).

Molecular basis of resistance to Moniliosis in apple

The molecular basis of resistance to moniliosis in apple (*Malus domestica*) involves a complex network of biochemical pathways and defense-related gene activation in response to *Monilinia* spp. infection (Hilber-Bodmer et

al. 2012). The initial stage of plant defense is triggered by the recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) located on the plant cell surface. This recognition activates PAMP-triggered immunity (PTI), leading to a cascade of intracellular signaling events (Martini et al. 2014).

One of the earliest responses is the rapid production of reactive oxygen species (ROS), known as the oxidative burst, which plays a dual role in directly inhibiting pathogen growth and acting as a signaling molecule to activate downstream defense mechanisms. This is followed by the induction of defense-related genes and the accumulation of pathogenesis-related (PR) proteins (Poniatowska et al. 2013).

Phytohormones play a central role in regulating these defense responses. Salicylic acid (SA) is primarily associated with resistance against biotrophic pathogens, while jasmonic acid (JA) and ethylene (ET) pathways are more involved in defense against necrotrophic pathogens such as *Monilinia* spp. In resistant apple cultivars, these signaling pathways are rapidly and efficiently activated, resulting in a stronger and more coordinated defense response (Fischer et al. 2017).

A key molecular mechanism underlying resistance is the activation of the phenylpropanoid pathway. This pathway leads to the synthesis of phenolic compounds, flavonoids, and lignin, which contribute to strengthening the plant cell wall and limiting pathogen invasion. Enzymes such as phenylalanine ammonia-lyase (PAL), peroxidase (POD), and polyphenol oxidase (PPO) play crucial roles in this process. Their increased activity results in the accumulation of antimicrobial compounds and the formation of a hostile environment for pathogen development (Fischer et al. 2017). Additionally, the reinforcement of the cell wall through lignification and callose deposition acts as a physical barrier against pathogen penetration. These molecular responses collectively restrict pathogen growth and confine infection to localized areas within the tissue (Van Brouwershaven et al. 2010).

Genetic basis of resistance to Moniliosis in apple

The genetic basis of resistance to moniliosis in apple is polygenic in nature and involves the interaction of multiple genes and quantitative trait loci (QTLs). Unlike monogenic resistance, which is controlled by a single gene, polygenic resistance provides a more stable and durable defense against pathogens such as *Monilinia* spp. (Poniatowska et al. 2013).

Resistance is largely determined by the expression of defense-related genes, including those encoding enzymes involved in secondary metabolite production, transcription factors, and proteins associated with signal transduction. Among these, transcription factors from the MYB, WRKY, and bHLH families play a significant role in regulating gene expression during pathogen attack. For example, the MdMYB10 gene is involved in the regulation of anthocyanin biosynthesis and phenylpropanoid metabolism, both of which contribute to enhanced defense responses (Poniatowska et al. 2013).

Quantitative trait loci associated with resistance have been identified in different apple genotypes, indicating that multiple genomic regions contribute to the overall resistance phenotype. These loci control various aspects of plant defense, including pathogen recognition, signal transduction, and activation of downstream defense responses (Förster & Adaskaveg 2000).

Molecular marker technologies such as SSR (Simple Sequence Repeat) and SNP (Single Nucleotide Polymorphism) markers are widely used to identify and map resistance-related genes and QTLs. Marker-assisted selection (MAS) enables breeders to efficiently select resistant genotypes at early stages of plant development, reducing the time and cost required for conventional breeding (Riccioni & Valente 2015).

Furthermore, genetic variability among apple cultivars plays a crucial role in determining their resistance levels. Resistant genotypes typically exhibit higher expression levels of defense genes and more efficient

activation of defense pathways, whereas susceptible genotypes show delayed or weaker responses (Akhoon et al. 2023).

In conclusion, the genetic basis of resistance to moniliosis in apple is governed by complex interactions among multiple genes and regulatory elements. Understanding these genetic mechanisms is essential for the development of resistant cultivars and the implementation of sustainable disease management strategies (Förster & Adaskaveg 2000).

Materials and Methods

Sampling

The study was conducted to evaluate the resistance of local apple cultivars (*Malus domestica*) to moniliosis caused by *Monilinia fructicola*. Four local apple cultivars widely in the Quba-Khachmaz region of Azerbaijan—Gandi-Sinab, Cirhaji, Gizil Ahmedi, and Sari-tursh—were used as research material. For each cultivar, three healthy fruit samples without visible disease symptoms were selected. The selected samples were artificially inoculated under laboratory conditions exclusively with *Monilinia fructicola*. The inoculated fruits were incubated under appropriate temperature and humidity conditions to ensure disease development.

Fungal isolation

The inoculum was prepared based on available positive samples of *Monilinia fructicola* maintained in the laboratory. Spore suspension was obtained from infected material and adjusted to a standardized concentration. After the appearance of disease symptoms, small tissue samples were taken from infected parts of the fruits. These samples were aseptically transferred onto Potato Dextrose Agar (PDA) medium in Petri dishes.

Microscopic analysis

Microscopic analysis confirmed the identification of the pathogen and allowed evaluation of structural differences among the colonies.

Real-time PCR amplification

DNA was isolated from infected plant tissues using standard laboratory methods. The presence of *Monilinia fructicola* was determined using real-time PCR with species-specific primers. The amplification process was carried out under optimized conditions, and Ct (cycle threshold) values were recorded. Ct values indirectly reflected the amount of pathogen in the samples. Lower Ct values indicated a higher infection level, whereas higher Ct values indicated a lower pathogen load.

Results and Discussion

The results of the study showed that although the response of apple cultivars to *Monilinia fructicola* varied among genotypes, the overall level of infection was not high.

In the Sari-tursh cultivar, no disease symptoms were observed after artificial inoculation, and the pathogen was not detected by real-time PCR analysis. This indicates that this cultivar is highly tolerant or resistant.

In the Gizil Ahmedi and Cirhaji cultivars, real-time PCR results showed Ct values above 30, indicating a low concentration of the pathogen in these samples. Disease symptoms were weakly expressed in these cultivars, and pathogen development remained limited.

In the Gandi-Sinab cultivar, the Ct value of approximately 28 indicated a relatively higher pathogen load compared to the other cultivars; however, this value does not correspond to strong susceptibility. Disease symptoms were observed in this cultivar, but the progression of infection was at a moderate level.

Observations on Petri dishes supported these findings. Although fungal growth was detected in all cultivars, the overall colony growth intensity was not high. Microscopic analysis revealed the presence of sporulation; however, this process was not highly intensive.

Overall, the obtained results indicate that the studied apple cultivars do not exhibit high susceptibility to *Monilinia fructicola*, and infection develops relatively weakly in all cultivars. The differences among cultivars are mainly reflected in pathogen quantity and the degree of symptom expression. Based on these findings, the cultivars can be conditionally classified as follows:

- More resistant (or highly tolerant): Sari-tursh;
- Moderately resistant: Gizil Ahmedi and Cirhaji;
- Relatively more susceptible (but not highly): Gandi-Sinab.

These results suggest that local apple cultivars possess a general background of resistance to moniliosis, and pathogen development is, in most cases, limited.

Conclusion

The present study demonstrated that local apple cultivars from the Quba-Khachmaz region exhibit varying responses to *Monilinia fructicola*, although the overall level of infection remained relatively low. Among the studied cultivars, Sari-tursh showed no detectable infection and can be considered highly tolerant or resistant.

Gizil Ahmedi and Cirhaji cultivars exhibited low pathogen loads, as indicated by higher Ct values, and showed limited symptom development, suggesting moderate resistance. In contrast, the Gandi-Sinab cultivar showed relatively higher pathogen presence, with moderate symptom expression; however, it did not exhibit strong susceptibility.

The integration of visual assessment, pathogen cultivation on nutrient media, microscopic observations, and real-time PCR analysis provided a comprehensive evaluation of disease development. The results indicate that local apple cultivars possess a general background tolerance to *Monilinia fructicola*, with differences primarily in the degree of pathogen accumulation and symptom expression. At the same time, the applied comprehensive approach can be recommended as an effective method for evaluating disease resistance in fruit crops..

Conflict of Interest

Authors declare that there is no conflict of interest.

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