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STAGE-DEPENDENT MORPHOPHYSIOLOGICAL AND BIOCHEMICAL RESPONSES REVEAL DROUGHT-ADAPTED WINTER WHEAT GENOTYPES

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Abstract: The drought responses of six modern winter wheat varieties and 15 stabilized F7 lines grown under rainfed conditions were evaluated using morphophysiological and biochemical traits. Relative water content, soluble protein content, enzyme activities, plant height, heading time, and yield were assessed at different developmental stages. Gobustan, Yubiley 90, and several F7 lines were identified as early heading genotypes, while a group of varieties and lines showed medium plant height associated with favorable productivity. Drought escape and tolerance mechanisms contributed positively to yield performance. Yubiley 90, Erytrospermum 100, 21 FAWWON9/Gaudio, 29th SAWYT N395, 21 FAWWON9/Gallio, and Gobustan/Gyrmyzy gul 1 were identified as promising genotypes for breeding. Gobustan/Gaudio, characterized by strong antioxidant defense, may serve as a donor for improving physiological drought tolerance.
Keywords: wheat, drought, productivity, antioxidant defense system, metabolic enzymes.

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Introduction

Drought tolerance is a complex trait governed by plant–environment interactions and therefore requires integrated evaluation. With the increasing frequency of drought under climate change, improving drought tolerance in winter wheat has become a major breeding priority. Selection based solely on grain yield may be insufficient; combining morphological, physiological, and biochemical traits can provide a more reliable assessment. Drought adversely affects wheat throughout vegetative and reproductive development, including germination, heading, flowering, and grain filling. Traits such as high tillering capacity, early maturity, a strong root system, moderate plant height, elongated upper internodes, and optimized leaf area contribute to drought adaptation by limiting water loss and maintaining plant viability. Physiological responses include stomatal closure and reductions in photosynthesis, transpiration, stomatal conductance, and relative water content, together with cell-wall modification, enhanced oxidative stress, and activation of alternative mitochondrial electron transport

pathways (Chaouachi et al. 2023). At the biochemical level, the efficiency of the Rubisco enzyme and photochemical efficiency decline; antioxidant defense systems are activated; abscisic acid, proline, polyamines, carbohydrates, and stress proteins accumulate (Nezhadahmadi et al. 2013).

Drought stress greatly influences photosynthesis in plants, resulting in decreased photosynthetic activity and negatively impacting plant development and productivity (Karami et al. 2025). As a C3 plant, wheat exhibits lower photosynthetic efficiency under water-deficient conditions compared to C4 plants. Studying the role of C4 photosynthetic enzymes, which are crucial for initial CO_2 fixation and the metabolism of carbon acids, under stress conditions in C3 plants is highly relevant. C4 photosynthetic enzymes play an important role in the non-photosynthetic organs of C3 plants by participating in the Krebs and Calvin cycles. They contribute to the biosynthesis of amino acids and help meet the NADPH demand of the antioxidant system.

Under drought stress conditions, the metabolic balance in plants is disrupted, leading to an increased demand for energy and, consequently, an enhancement of defense mechanisms. In such situations, AAT contributes to amino acid synthesis and the redistribution of nitrogen, thereby promoting the production of osmoprotectants, maintaining energy balance, and preventing the accumulation of harmful compounds during stress.

Metabolites produced via phosphoenolpyruvate carboxylase (PEPC) contribute to the reduction of oxidative stress and the detoxification of ROS. PEPC supports the metabolic adaptation of plants under drought conditions, maintains carbon and energy balance, and enhances drought stress tolerance (Li et al. 2024).

Under water-deficit conditions, a significant increase in the activity of C4 photosynthetic enzymes in wheat has been demonstrated in studies conducted. In genotypes with high enzyme activities, photosynthetic efficiency, stress tolerance, and metabolic plasticity are enhanced. Under drought conditions, due to the reduction in photosynthesis, NAD-MDH contributes to mitochondrial respiration by synthesizing ATP and NADH, thereby enhancing the activity of antioxidant enzymes and mitigating oxidative stress (Kambona et al. 2023).

The study aimed to evaluate the relationship between certain morphophysiological and biochemical traits under drought conditions in winter bread wheat and to screen for tolerant genotypes.

Materials and Methods

Experimental site and plant material

The study was conducted under rainfed conditions at the Gobustan Experimental Station of the Research Institute of Crop Husbandry, Ministry of Agriculture of the Republic of Azerbaijan. The site is located at an altitude of 800 m above sea level. Twenty-one bread wheat (*Triticum aestivum* L.) genotypes were used as study materials. The genotypes Gaudio and Gallio originated from Austria, Lutescens 76 and Erytrospermum 100 from Russia, while the remaining genotypes were of Azerbaijani origin.

Planting and experimental design

The seeds of bread wheat genotypes were planted at the end of October, at an average density 400 seeds per m^2, at 1m x 10 m plots, consisting of 7 rows placed 15 cm apart. During the vegetation period, phenological observations were conducted, plant height was measured at full maturity, and grain productivity was determined for each genotype.

Productivity

The harvested spikes from a 1 m² area were threshed using a threshing machine, the grains were separated and weighed. The mean value of three replications was calculated, and the resulting value was considered as the yield per 1 m² (g/m²).

Plant height

Before harvesting, the height of 10 plants in each plot was measured, and the average value was used to determine the plant height for each genotype (cm).

Determination of Relative Water Content

Relative water content (RWC) in leaves was measured based on the method proposed by Turner (1981) and calculated using the following formula:

$$RWC = [(FW - DW) / (TW - DW)] \times 100$$

where: FW – fresh weight of the leaf, TW – turgid weight, DW – dry weight.

Preparation of enzyme extract

A 0.5 g leaf sample was ground in liquid nitrogen and homogenized in 100 mM sodium phosphate buffer (pH 7.8) containing 1 mM EDTA, 2 mM PMSF, 1% PVP, and 0.1% Triton X-100. The homogenate was then centrifuged, and the resulting supernatant was used for the analysis of antioxidant enzyme activities. Enzyme activity was calculated based on the following formula:

$$A = \Delta OD \cdot V / \varepsilon \cdot b \cdot \tau$$

A – activity in the International System of Units; ΔOD – change in optical density per minute; V – volume of the reaction mixture (ml); τ – time of the reaction; ε – molar extinction coefficient, b – volume of the enzyme extract added to the reaction mixture (µl). The specific activities of the enzymes were calculated relative to protein content and per 1 g of fresh weight.

Extraction of metabolic enzymes from leaf samples

Leaf samples were homogenized in 100 mM Tris-HCl (pH 7.8) containing 5 mM DTT, 5 mM $MgCl_2 \cdot 6H_2O$, 1 mM EDTA, 0.5% Triton X-100, and 1% PVP. The homogenate was centrifuged at 1000g for 10 minutes and then at 5000g for 30 minutes at 4°C. The activity of enzymes was determined by measuring the decrease in absorbance at 340 nm resulting from NADH oxidation within 1 min (Ultrospec 3300 spectrophotometer). Spectrophotometric measurements were carried out in 1.0 ml cuvettes.

Aspartate aminotransferase (AAT, EC 2.6.1.1) activity assay

AAT activity was determined according to the method of Yang et al. (2019). For the assay, the reaction mixture contained 100 mM Tris-HCl buffer (pH 8.5), 2 mM EDTA, 2.5 mM 2-oxoglutarate, 10 µg/ml pyridoxal phosphate, 10 mM DTT, 12 U/ml MDH and 0.2 mM NADH. To this mixture, 20 µl of leaf extract and 2.5 mM L-aspartate were added. The activity was calculated using a molar extinction coefficient of ε = 6.22 $mM^{-1} \cdot cm^{-1}$ and expressed as µmol aspartate mg^{-1} protein min^{-1}.

NADP-malic enzyme (NADP-ME, EC 1.1.1.40) activity assay

NADP-ME activity was determined by the method of Arias et al. (2018). The reaction mixture consisted of 2.5 mM Tris-HCl buffer (pH 8.3), 0.5 mM EDTA, 0.75 µM CoA, 0.25 mM NADP, 2.5 mM malate, and 5.0 mM $MnCl_2$. The activity was calculated using a molar extinction coefficient of ε = 6.22 $mM^{-1} \cdot cm^{-1}$ and expressed as µmol malate mg^{-1} protein min^{-1}.

Malate dehydrogenase (NAD-MDH, EC 1.1.1.38) activity assay

The activity of NAD-MDH was determined according to the method of

Aliyeva et al. (2023). The reaction mixture consisted of 100 µM Tris-HCl buffer (pH 8.0), 1 mM oxaloacetic acid (OAA), 10 mg/ml bovine serum albumin (BSA), 10 mM $MgCl_2$, 0.15 µM NADH, and 10 µl of enzyme extract. The activity was calculated using a molar extinction coefficient of ε = 6.22 $mM^{-1} \cdot cm^{-1}$ and expressed as µmol OAA mg^{-1} protein min^{-1}.

Phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31) activity assay

PEPC enzyme activity was determined according to the method of Aliyeva et al. (2023). The reaction mixture consisted of 60 mM HEPES-NaOH buffer (pH 8.0), 10 mM $MgCl_2$, 2 mM DTT, 10 mM $NaHCO_3$, 0.2 mM NADH, 10 units of MDH, 10 mM phosphoenolpyruvate, and 40 µl of enzyme extract. The activity was calculated using a molar extinction coefficient of ε = 6.22 $mM^{-1} \cdot cm^{-1}$ and expressed as µmol PEP mg^{-1} protein min^{-1}.

Determination of the amount of soluble protein

Protein content was determined spectrophotometrically at 595 nm according to the method proposed by Bradford (1976). During the construction of the calibration curve, different concentrations of bovine serum albumin (BSA) diluted in water were used as the standard protein.

Statistical analysis

Data from three independent biological replicates were analyzed using one-way analysis of variance (ANOVA). When significant differences were observed, Tukey's Honest Significant Difference (HSD) post hoc test was applied for pairwise comparisons.

Results

Drought is one of the environmental factors that negatively affects plant development at the early stages of vegetative growth, ultimately leading to a reduction in productivity. In this study, we determined a range of morphophysiological and biochemical parameters in 21 bread wheat genotypes cultivated under rainfed conditions at the Gobustan Experimental Station of the Research Institute of Crop Husbandry of the Azerbaijan Republic.

In the 2023–2024 vegetation period, plant heights of the genotypes were measured before harvest, and the results are presented in Fig. 1A. Statistical analysis revealed significant differences in plant height among the genotypes at the 0.01 significance level (CV 2.7%, LSD 4.3**). As shown in the figure, the plant height of the studied genotypes varied widely, ranging from 83 to 115 cm, with an average value of 97.3 cm. Among the studied genotypes, the tallest plant heights were observed in Gobustan/Gyrmyzy gul 1 (115 cm) and Gobustan/Gallio (110 cm). The shortest plant heights were recorded in the varieties Yubiley 90, Gyrmyzy gul 1, and Gaudio (96, 95, and 95 cm, respectively), and in the lines Quality/Balaton, Lutescens 76, 21FAWWON61/Balaton, Erytrospermum 100, and Gobustan/AZE026-10-4 (95, 92, 85, 84, and 83 cm, respectively). The remaining genotypes exhibited intermediate plant heights.

At the same time, the yield performance of the studied genotypes was evaluated, and the results are presented in Fig. 1B. The analysis of variance revealed a statistically significant difference among the genotypes for this parameter at the 0.01 significance level (CV 4.5%, LSD 40.6**).

As shown in the Fig. 1B, the yield of the varieties Lider and Yubiley 90 was 726 and 709 g/m², respectively, which was higher than that of the other genotypes. Among the studied lines, Erytospermum 100 (625 g/m²), Gobustan/Balaton (592 g/m²), and 29th SAWYT N~~o.~~ 395 (612 g/m²) demonstrated high performance. In terms of yield, the lowest values among the varieties were observed in Gobustan and Gallio, while among the lines, Gobustan/AZE 026-

10-4, Gyrmyzy gul 1/Gobustan, Lutescens 76, Quality/Balaton, Gobustan/Gallio, Gobustan/Gaudio and Quality/Gaudio 1 exhibited the lowest results. The remaining genotypes showed intermediate yield performance. Thus, along with other parameters, the yield data of genotypes should also be considered in the continuation of the breeding program.

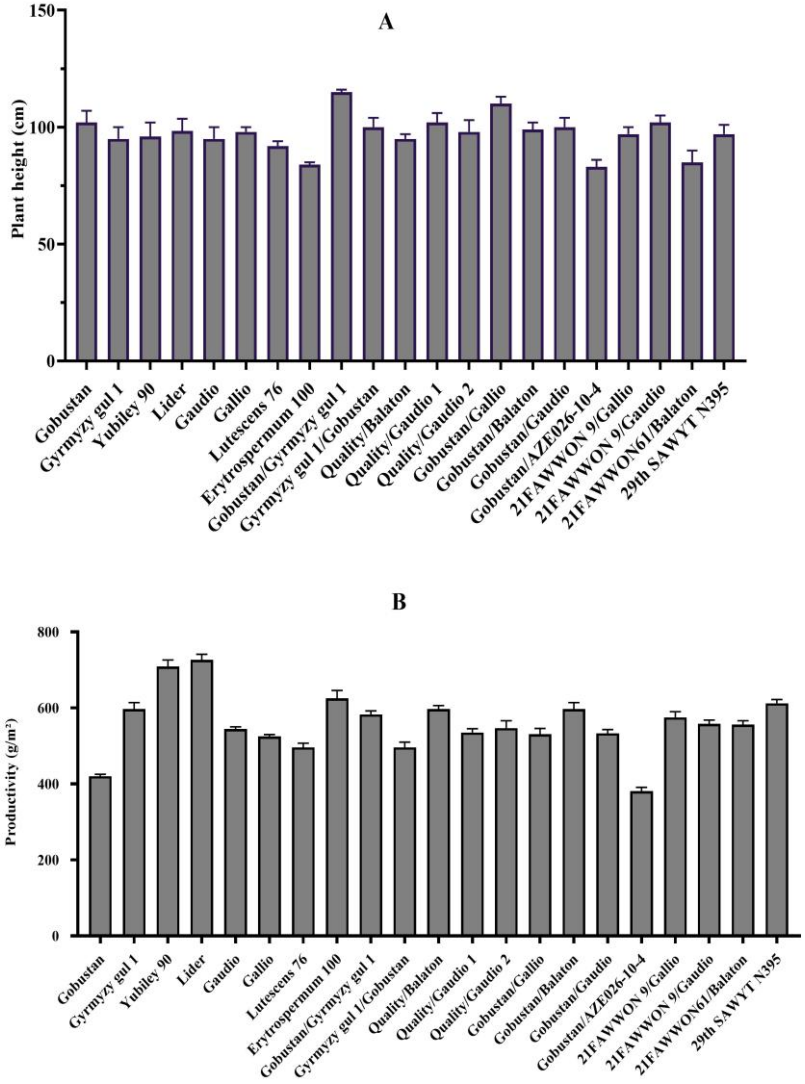

Figure 1. Plant height of the studied genotypes: CV 2.7%, LSD 4.3** (A); Productivity of the studied genotypes: CV 4.5%, LSD 40.6** (B). The data are presented as the means ± SD and are obtained from 3 biological replicates.

Heading dates for the studied genotypes in the 2023–2024 vegetation period are presented in Table. As shown in the table, the heading dates of the genotypes ranged from April 29 to May 7. Considering that the heading date is an important phenological trait of wheat genotypes and plays a significant role in productivity under

rainfed conditions, it was recorded as the number of days from May 1, and statistical analysis was carried out accordingly. The analysis of variance revealed statistically significant differences among genotypes for this parameter. Among the studied varieties, Gobustan and Yubiley 90 exhibited early heading on April 30 and May 1, respectively, while Gaudio, Gyrmyzy gul 1, Gallio and Lider were classified as late-heading varieties (Table). Among the lines, Gyrmyzy gul 1/Gobustan, 21FAWWON61/Balaton, Gobustan/ Gyrmyzy gul 1 and Quality/Gaudio 1 exhibited early heading. In contrast, 29th SAWYT N395 and 21FAWWON9/Gaudio showed late heading. It should be noted that the late-heading varieties mentioned above were developed for irrigated and moisture-sufficient rainfed regions.

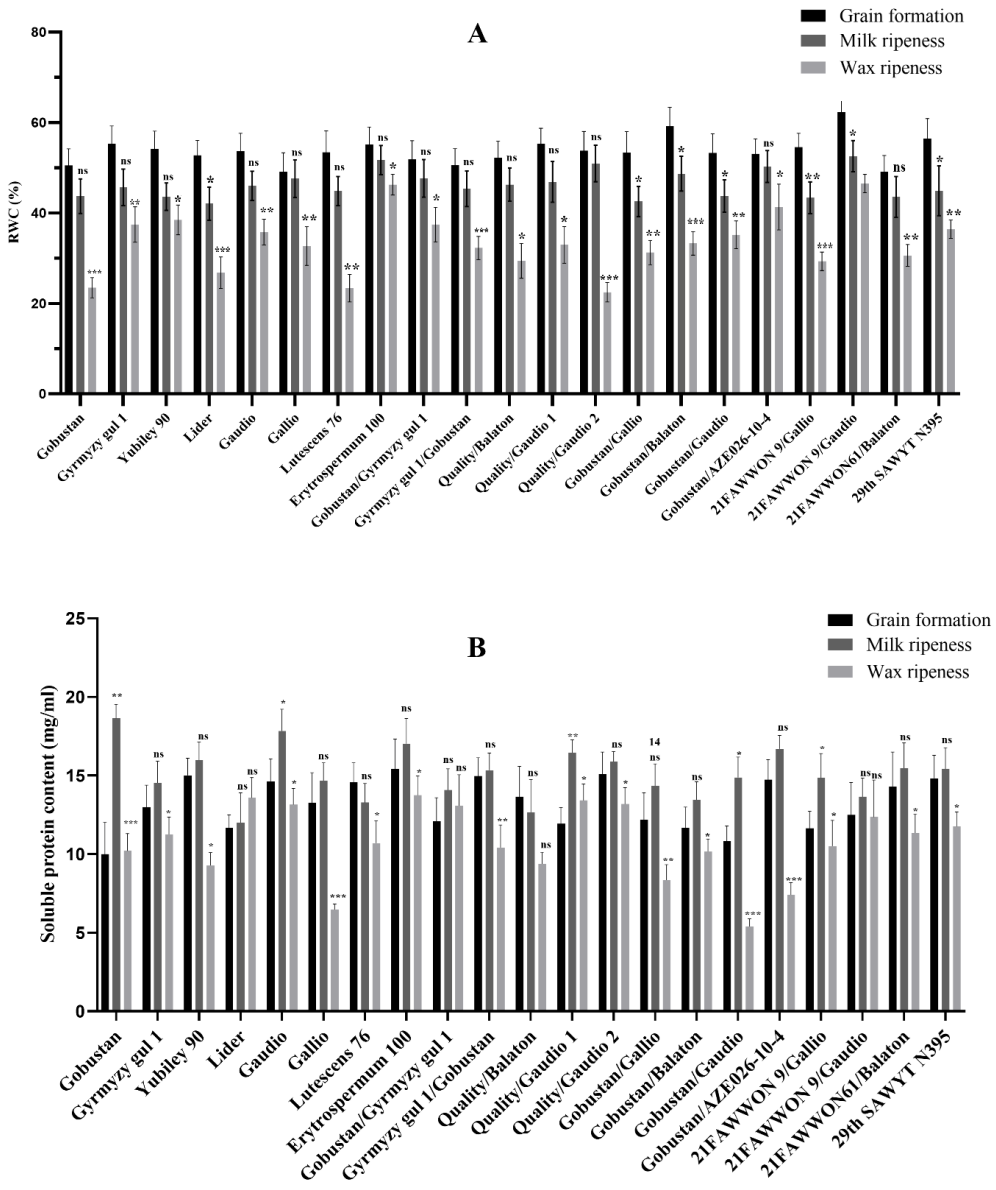

Figure 2. Relative water content (A), and soluble protein content (B) in bread wheat genotypes. Statistical significance was set at $p < 0.05$ (*, significant) and $p < 0.01$ (**, very significant), $p < 0.001$ (***, highly significant); results with $p \geq 0.05$ were considered not significant (ns).

From the perspective of heading time, the early spike emergence of Gobustan and Yubiley 90 varieties makes them more suitable for cultivation in arid rainfed regions, where drought stress typically occurs in the later stages of the vegetation period. On the other hand, the early-heading characteristics of lines such as Gyrmyzy gul 1/Gobustan, 21FAWWON61/Balaton, Gobustan/ Gyrmyzy gul 1, and Quality/Gaudio 1 suggest their potential use in breeding programs targeting moisture-deficient rainfed environments.

The results of relative water content (RWC) measurements of the flag leaf during the grain formation (May 21, 2024), milk ripeness (May 30, 2024), and wax ripeness (June 11, 2024) stages for the studied genotypes in the vegetation period are presented in Fig. 2A.

The analysis of variance revealed statistically significant differences among genotypes for this parameter. As shown in the table, the RWC of the flag leaf varied between 49.1% and 62.3% (CV 13.2%) during the grain formation stage, between 42.1% and 52.6% (CV 10.5%) during the milk ripeness stage, and between 22.5% and 46.3% (CV 23.8%) during the wax ripeness stage.

As seen from the presented results, during the grain formation and milk ripeness stages, the variation in RWC was within a narrower range due to the presence of a certain level of soil moisture and the relatively young physiological state of the genotypes. In other words, the genotypes showed less differentiation in this parameter during these stages. However, in the wax ripeness stage, the reduction in soil moisture led to a more pronounced expression of differences among genotypes. Thus, at this stage, the RWC values in the lines 21FAWWON 9/Gaudio and Erytrospermum 100 were considerably higher than those of other genotypes (46.5% and 46.3%, respectively), indicating their better regulation of water balance under conditions of declining soil moisture. In contrast, the lowest RWC values at this stage were recorded in the line Quality/Gaudio 2 (22.5%), the line Lutescens 76 (23.4%), and the variety Gobustan (23.5%), suggesting water regime regulation in these genotypes.

Thus, the study of RWC in several wheat varieties and lines revealed significant differences among genotypes for this parameter and it is advisable to use genotypes with higher flag leaf RWC in future breeding programs.

The results of the analyses showed that reduced water content in plants significantly decreases the amount of proteins in the cells. In the samples taken during the initial stage, the amount of soluble proteins ranged between 10.00 mg/ml and 15.41 mg/ml (Fig. 2B).

The AAT enzyme is one of the key regulatory enzymes that enhance plant tolerance under drought conditions (Fig. 3A). High activity levels of this enzyme were observed in the Erytrospermum 100 and Gobustan/Gaudio genotypes during the studied growth phases. According to the study results, the lowest activity levels were recorded in the Gobustan/Gallio genotype and in the Lutescens 76 genotype. In the parental varieties, Gobustan, Gyrmyzy gul 1, Yubiley 90, Gaudio, and Gallio, enzymatic activity remained at a moderate level. However, some hybrid forms, such as Gyrmyzy gul 1/Gobustan and Quality/Gaudio 2, exhibited higher enzyme activity compared to their parental lines.

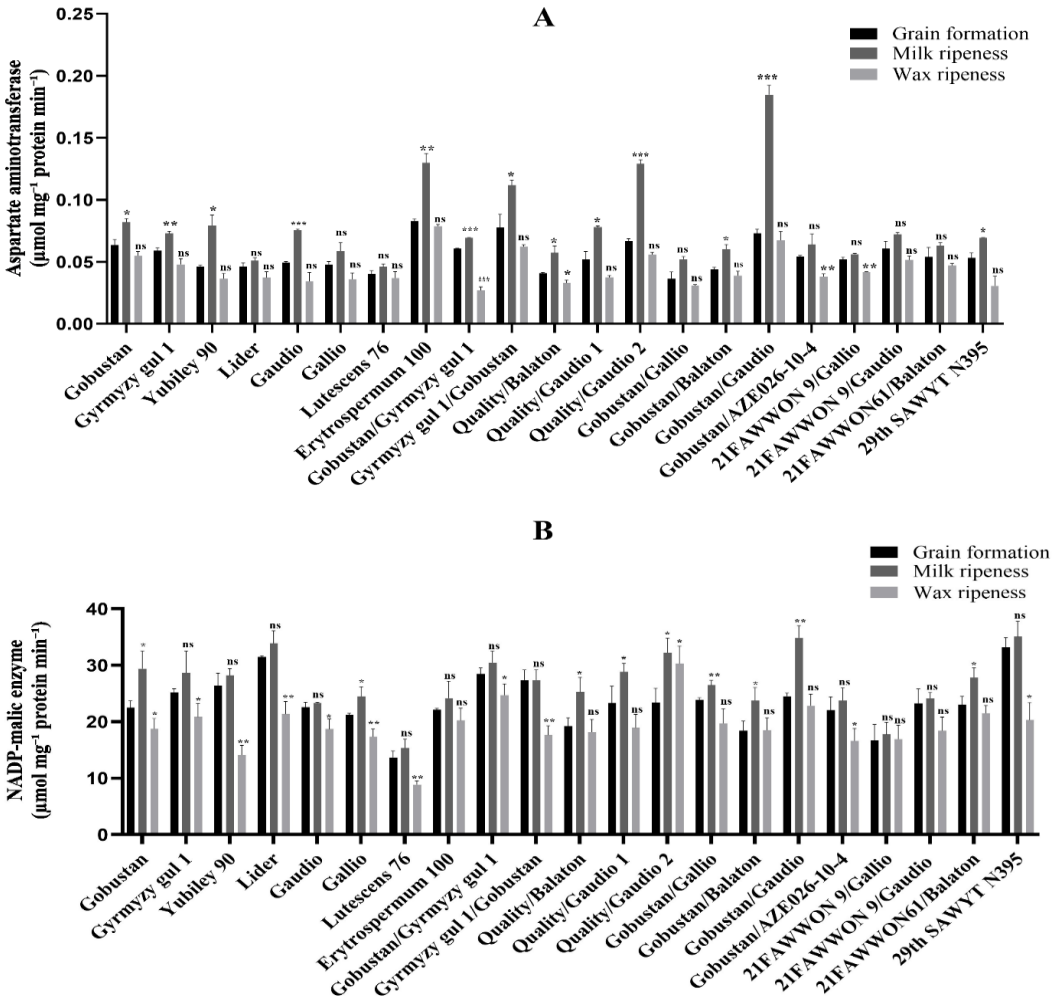

Figure 3. Activity of AAT (A) and NADP-ME (B) in bread wheat genotypes. Statistical significance was set at $p < 0.05$ (*, significant) and $p < 0.01$ (**, very significant), $p < 0.001$ (***, highly significant); results with $p \geq 0.05$ were considered not significant (ns).

In general, the enzyme activity was highest during the milk ripeness stage. At this stage, the highest AAT activity was recorded in the Gobustan/Gaudio genotype. This value differed significantly from those of the other genotypes in statistical terms ($p \leq 0.05$).At the wax ripeness stage, AAT activity decreased in almost all genotypes, indicating a decline in enzyme activity toward the later stages of development. The results of the study revealed that the activity of AAT in bread wheat genotypes varied significantly depending on the phenological stage and genotype. The Gobustan/Gaudio and Erytrospermum 100 genotypes exhibited high enzymatic activity across all stages of the study. In contrast, the Gobustan/Gallio and Lutescens 76 genotypes showed relatively low activity. Notably, in hybrid forms, a marked increase in activity was observed, particularly during the milk ripeness stage.

The 29th SAWYT N395 hybrid exhibited the highest NADP-ME activity across all developmental stages (Fig. 3B). High NADP-ME activity was also observed in the Lider, Gobustan/Gaudio, and Quality/Gaudio 2 specimens, whereas the lowest enzymatic activity was recorded in Lutescens 76 and 21FAWWON 9/Gallio.

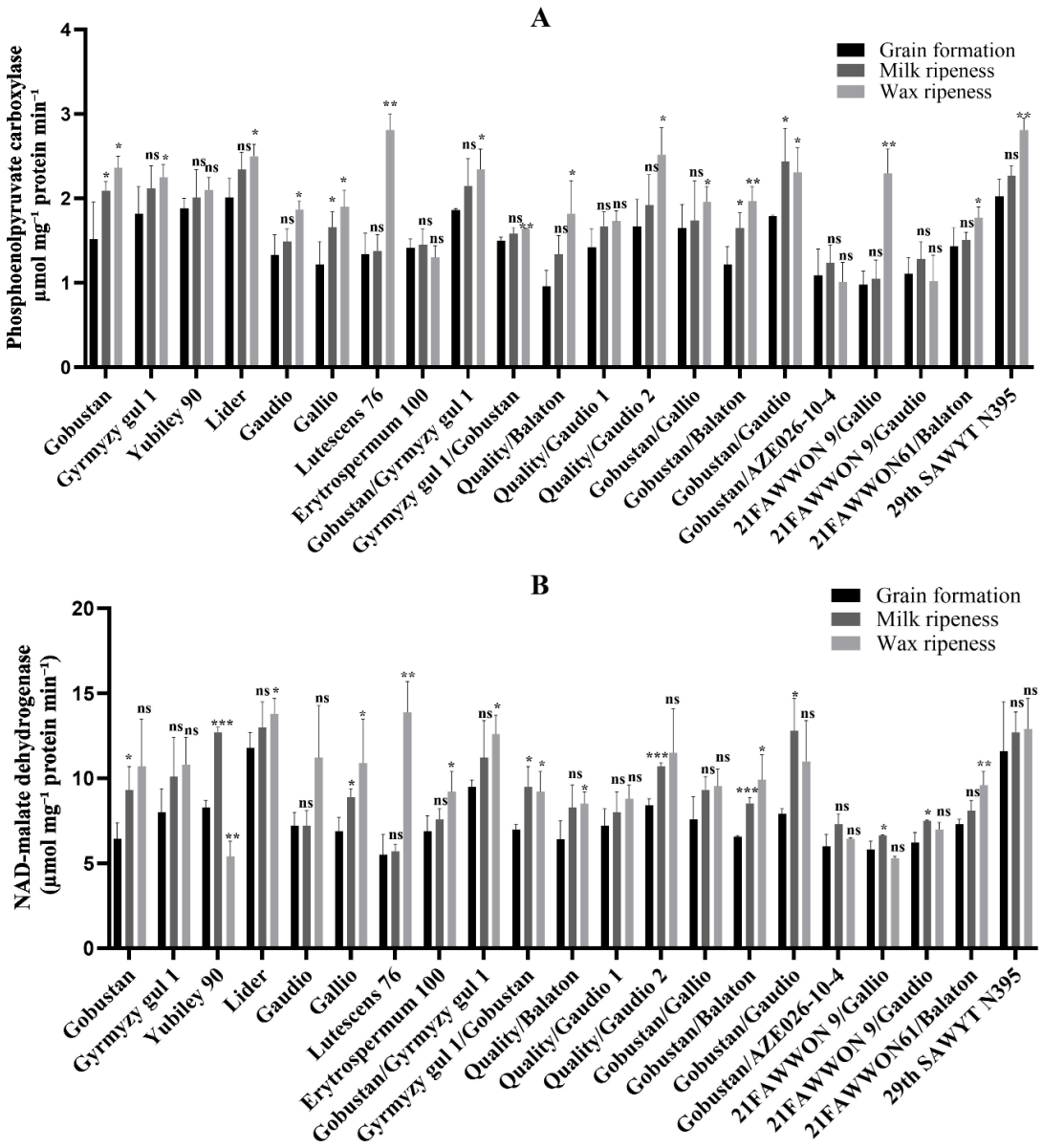

Figure 4. Activity of PEPC (A) and NAD-MDH (B) in bread wheat genotypes. Statistical significance was set at $p < 0.05$ (*, significant) and $p < 0.01$ (**, very significant), $p < 0.001$ (***, highly significant); results with $p \geq 0.05$ were considered not significant (ns).

The results of the study demonstrated that the activity of PEPC in bread wheat genotypes varied significantly depending on the phenological development stages and genotypes (Fig. 4A). In general, the highest enzyme activity was observed during the wax ripeness stage. At this stage, the 29th SAWYT N395 and Lutescens 76 genotypes exhibited enzyme activity levels of 2.9 and 2.8 µmol mg^{-1} protein min^{-1}, respectively, which were statistically significantly higher than those of all other genotypes. In addition, Quality/Gaudio, Gobustan/Gaudio, and Yubiley 90 genotypes also showed high PEPC activity. The lowest activity was recorded in the 21FAWWON 9/Gaudio, 21FAWWON 9/Gallio, and 21FAWWON61/Balaton genotypes,

particularly during the f grain formation and milk ripeness stages. High NAD-MDH activity was observed in the 29th SAWYT N395 and Gobustan/Gyrmyzy gul 1 genotypes (Fig. 4B). These genotypes not only exhibited strong metabolic activity but also showed increasing enzyme levels throughout the vegetation period. These characteristics indicate their potential for high productivity and tolerance to abiotic stress. The lowest NAD-MDH activity was recorded in the 21FAWWON 9/Gallio hybrid.In the Yubiley 90 variety, a sharp decrease in enzyme activity was observed during the wax ripeness stage. Although NAD-MDH activity in the Lutescens 76 genotype was low during the grain formation and milk ripeness stages (5.5 and 5.7 µmol mg^{-1} protein min^{-1}, respectively), it increased during the wax ripeness stage, reaching its maximum level (13.9 µmol mg^{-1} protein min^{-1}). In the Gobustan/Gaudio and Quality/Gaudio2 hybrids, an increase in enzyme activity was also observed during the wax ripeness stage. These results indicate that these genotypes possess a more stable and progressively adaptive metabolic mechanism.

The heatmap analysis revealed that the increase in PEPC and NADP-ME activities observed in tolerant genotypes indicates enhanced metabolic flexibility, supporting carbon fixation and energy supply under drought stress (Fig. 5).

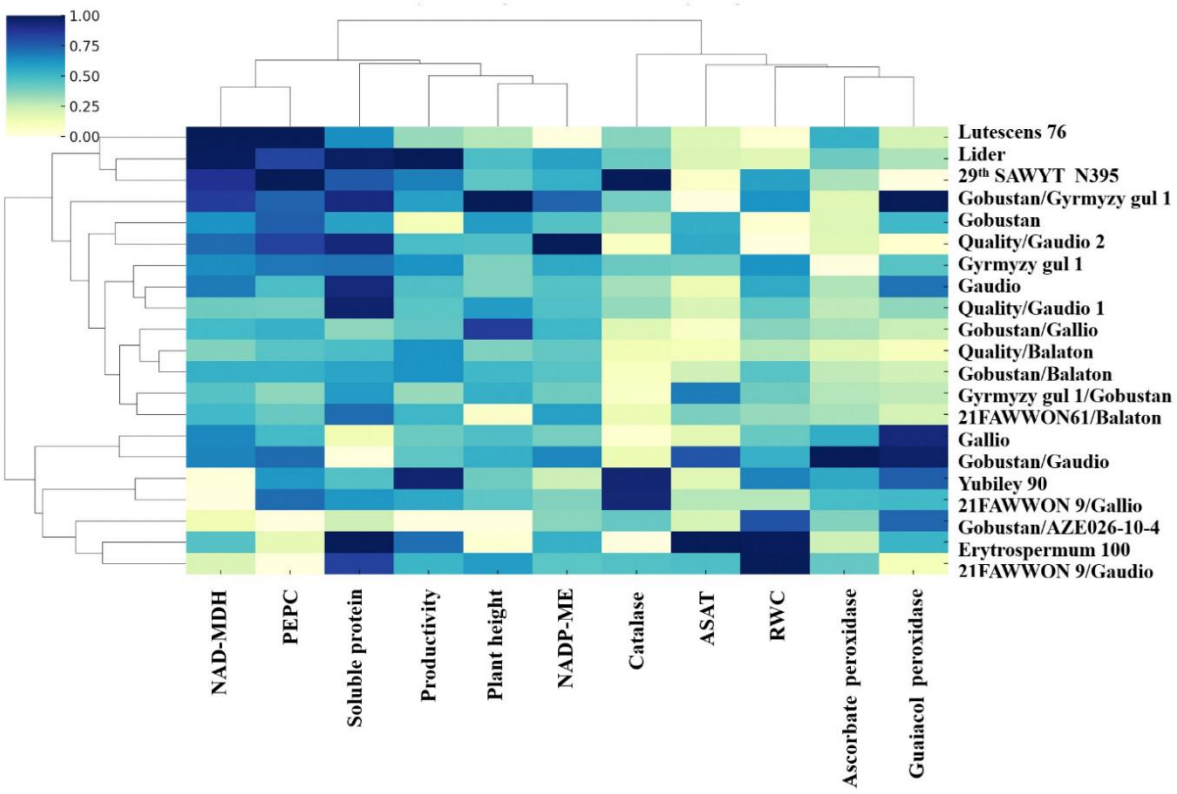

Figure 5. Clustered heatmap with dendrograms - genotypes are now grouped according to their overall biochemical and physiological similarity.

Based on the overall analysis, the investigated genotypes were grouped into three main clusters (Fig. 5). Cluster 1 included Yubiley 90 and Lider, which were characterized by high productivity and strong antioxidant enzyme activities.

Cluster2 comprised Gobustan and Gobustan/AZE026-10-4 genotypes, which exhibited relatively lower productivity but showed high RWC and strong stress adaptation capacity, indicating their potential drought tolerance. Cluster 3 consisted of genotypes with intermediate and diverse characteristics, including Gyrmyzy gul 1, Gaudio, Gallio, Lutescens 76, Erytrospermum 100, Gobustan/Gyrmyzy gul 1, Gyrmyzy gul 1/Gobustan, Quality/Balaton, Quality/Gaudio 1, Quality/Gaudio 2, Gobustan/Gallio, Gobustan/Balaton, Gobustan/Gaudio, 21FAWWON 9/Gallio, 21FAWWON 9/Gaudio, and 21FAWWON61/Balaton. The genotypes in this group exhibited a wide range of productivity and enzyme activity levels, representing valuable genetic material for future breeding programs aimed at improving both yield potential and stress tolerance.

Discussion

Drought stress negatively affects wheat productivity by causing changes at morphological, physiological, and biochemical levels (Ayed et al. 2021). Under rainfed conditions with limited moisture availability, plant height plays a significant role in both drought tolerance and productivity in wheat.

As a partial drought escape mechanism, heading time plays a crucial role in yield formation under rainfed conditions. The timing of heading is not only a genotypic trait specific to the plant, but also depends on the hydrometeorological factors of the vegetation period. According to the literature, in regions where drought tends to occur during the later stages of vegetation, early heading and flowering can serve as mechanisms to avoid drought. Several studies have also emphasized that the heading and fertilization stages are among the most drought-sensitive phases in wheat (Dong et al. 2017).

RWC is an important indicator of drought tolerance that ensures metabolic activity in plant tissues under water-deficient conditions. This parameter tends to be high during the early stages of plant development but decreases as the plant's dry biomass begins to increase. Although a plant's water potential depends on the water status of the soil, plant, and atmosphere, leaf RWC specifically reflects the plant's internal water status, making it a more accurate indicator of water balance in the plant. A decline in RWC in response to drought stress has been observed in various plant species. Several studies have also reported genotypic differences in leaf RWC in wheat plants (Tanaka and Ito 2025). Leaf RWC is associated with drought tolerance and may be considered a better indicator of drought stress than other morphophysiological and biochemical parameters of plants. RWC reflects the proportion of water in a leaf relative to its full water-holding capacity at the time of measurement, and it serves as an indicator of the balance between water absorption from the soil and water loss through transpiration under identical conditions. Since transpiration typically occurs during midday hours, the RWC measured at this time depends on the plant's ability to absorb and retain water from the soil. Therefore, midday RWC values represent the plant's overall capacity for water uptake and evaporation, while also characterizing the water deficit in the leaf.

AAT is one of the key enzymes involved in the transamination of amino acids, and its activity is closely associated with the efficiency of nitrogen metabolism. The high activity of the enzyme during the milk ripeness stage indicates intensive protein synthesis and redistribution of nitrogen at this stage. This was particularly pronounced in the Gobustan and Ertyospermum 100 genotypes, suggesting

that these genotypes exhibit more efficient nitrogen metabolism.

In many cases, hybrid forms showed higher NADP-ME activity compared to their parental lines. These results reflect potential differences in the Krebs cycle and mitochondrial respiration. The NADP-ME enzyme has been studied in some C3 plants such as *Arabidopsis thaliana* and *Oryza sativa* (Liu 2007).

The NADP-ME enzyme also plays a key role in plant defense mechanisms against abiotic stresses such as water deficiency and high temperatures (Li et al. 2025). From this perspective, genotypes exhibiting high enzyme activity should be considered for selection programs as indicators of stress tolerance. Besides, the reaction catalyzed by NADP-ME results in the release of CO_2 (Sun et al. 2019), which can enter the Calvin cycle and contribute to an increase in photosynthetic products. In general, the presented results suggest that certain hybrid genotypes demonstrated higher enzymatic activity compared to their parental lines. This strengthens the likelihood that these hybrids are better adapted to abiotic stresses and enhances their potential for use in breeding programs.

PEPC catalyzes the conversion of PEP to oxaloacetic acid OAA, providing a link between carbohydrate and nitrogen metabolism (Wei et al. 2025). In our study, PEPC activity increased significantly during the wax ripeness stage. The 29th SAWYT N395 and Lutensens 76 genotypes, which showed high enzyme activity at this stage, probably indicated a greater need for energy supply and regulatory metabolism at the final stage of development. Since the wax ripeness stage represents the final phase of seed development, the increase in enzymatic activity may be associated with the redirection of carbon skeletons into biosynthetic processes. PEPC plays a crucial role in plant adaptation to abiotic stresses, including drought and salt stress. Increasing enzyme activity may support enhanced photosynthetic efficiency and anabolic processes (Chen et al. 2019). The lowest activity observed in the FAWWON series genotypes suggests a weaker metabolic response, indicating that these genotypes should be carefully evaluated in breeding programs. In contrast, the high enzyme activity observed in genotypes such as Quality/Gaudio and Gobustan/Gaudio indicates that they are metabolically more active and potentially more tolerant to stress conditions.

NAD-MDH catalyzes the conversion of malate to OAA and NADH, thereby acting as a bridge between carbon metabolism and energy production (de Lorenzo et al. 2024). In our study, the variation in NAD-MDH enzyme activity across developmental stages indicates that the enzyme is dynamically regulated in accordance with the plant's metabolic demands. In most genotypes, the peak activity observed during the wax ripeness stage is associated with the accumulation of energy and carbon reserves in the seed at this phase. This trend is particularly evident in the genotypes Lider, Lutescens 76, and 29th SAWYT N395, highlighting their high potential in energy metabolism. These genotypes are recommended for careful evaluation in breeding programs. Differences in NAD-MDH activity may also be linked to adaptation mechanisms under stress conditions. Studies have shown that this enzyme supports mitochondrial energy flux and enhances ROS balance during abiotic stresses, especially salt and drought stress (Monteiro-Batista et al. 2025).

Thus, the comprehensive research conducted indicates that the genotypes Yubiley 90, Erytrospermum 100, 21FAWWON 9/Gaudio, Gobustan/Gaudio, Gobustan/Gyrmyzy gul 1, 29th SAWYT N395, and 21FAWWON 9/Gallio

demonstrated notable drought tolerance and are considered suitable for cultivation in moisture-deficient arid regions. Among them, the Gobustan/Gaudio genotype, which possesses a strong antioxidant defense system, may serve as a valuable source material for antioxidant analysis and biochemical screening.

Conclusion

The results obtained provide a better understanding of the mechanisms underlying drought tolerance under rainfed conditions and help clarify the role of antioxidant enzymes in stimulating a broad spectrum of protective responses. The research revealed significant differences in drought tolerance levels among modern winter wheat varieties and lines. Based on the analysis of biochemical indicators, it was found that high RWC and sustained activity of antioxidant and metabolic enzymes are key traits contributing to better adaptation under drought conditions. Varieties possessing these traits are less affected by environmental stress factors and are able to maintain their productivity. The findings of this study enable the identification of promising genotypes for use in breeding programs. A comprehensive analysis of drought tolerance creates a scientific basis for more efficient breeding programs and the development of wheat varieties tolerant to climate change.

Conflict of Interest

Authors declare that there is no conflict of interest.

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References

Aliyeva D.R., Gurbanova U.A., Rzayev F.H., Gasimov E.K., Huseynova I.M. (2023) Biochemical and ultrastructural changes in wheat plants during drought stress. *Biochemistry*, 88(11):1944–1955. Doi: 10.1134/S0006297923110226

Arias C.L., Pavlovic T., Torcolese G., Badia M.B., Gismondi M., Maurino V.G., Andreo C.S., Drincovich M.F., Gerrard Wheeler M.C., Saigo M. (2018) NADP-Dependent malic enzyme 1 participates in the abscisic acid response in *Arabidopsis thaliana*. *Front Plant Sci*, 9:1637. Doi: 10.3389/fpls.2018.01637

Ayed S., Bouhaouel I., Othmani A., Bassi F.M. (2021) Use of wild relatives in durum wheat (*Triticum turgidum* L. var. *durum* Desf.) breeding program: Adaptation and stability in context of contrasting environments in Tunisia. *Agron*, 11:1782. Doi: 10.3390/agronomy11091782.

Bradford M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1–2):248–254. Doi: 10.1006/abio.1976.9999

Chaouachi L., Marín-Sanz M., Kthiri Z., Boukef S., Harbaoui K., Barro F., Karmous Ch. (2023) The opportunity of using durum wheat landraces to tolerate drought stress: screening morpho-physiological components, AoB. *Plants*, 15(3):plad022. Doi: 10.1093/aobpla/plad022

Chen Q., Wang B., Ding H., Zhang J., Li S.C. (2019) The role of NADP-malic enzyme in plants under stress. *Plant Sci*, 281:206–212. Doi: 10.1016/j.plantsci.2019.01.010.

de Lorenzo L., Stack T.M.M., Fox K.M., Walstrom K.M. (2024) Catalytic mechanism and kinetics of malate dehydrogenase. *Essays Biochem*, 68(2):73–82. Doi: 10.1042/EBC20230086

Dong B., Zheng X., Liu H., Able J.A., Yang H., Zhao H., Zhang M., Qiao Y., Wang Y., Liu M. (2017) Effects of drought stress on pollen sterility, grain yield, abscisic acid and protective enzymes in two winter wheat cultivars. *Front. Plant Sci*, 8:1008. Doi: 10.3389/fpls.2017.01008

Huseynova I.M., Rustamova S.M., Suleymanov S.Y., Aliyeva D.R., Mammadov A.C., Aliyev J.A. (2016) Drought-induced changes in photosynthetic apparatus and antioxidant components of wheat (*Triticum durum* Desf.) varietes. *Photosynth. Res*, 130(1-3):215–223. Doi: 10.1007/s11120-016-0244-z

Kambona C.M., Koua P.A., Léon J., Ballvora A. (2023) Stress memory and its regulation in plants experiencing recurrent drought conditions. *Theor Appl Genet*, 136(2):26. Doi: 10.1007/s00122-023-04313-1.

Karami S., Shiran B., Ravash R. (2025) Molecular investigation of how drought stress affects chlorophyll metabolism and photosynthesis in leaves of C3 and C4 plant species: A transcriptome meta-analysis. *Heliyon*, 11(3):e42368. Doi: 10.1016/j.heliyon.2025.e42368.

Li S., Zheng H., Sui N., Zhang F. (2024) Class III peroxidase: an essential enzyme for enhancing plant physiological and developmental process by maintaining the ROS level: A review. *International Journal of Biological Macromolecules*, 283: 137331. Doi: 10.1016/j.ijbiomac.2024.137331

Li H., Xiao J., Chen J., Shen X., Luo J., Guo F., Wang S., Xu L., Guo X., Wang S., Wang H., Wang W. (2025) Identification of the cassava NADP-ME gene family and its response and regulation in photosynthesis. *Front Plant Sci*, 16:1525193. Doi: 10.3389/fpls.2025.1525193

Liu S., Cheng Y., Zhang X., Guan Q., Nishiuchi Sh., Hase K., Takano T. (2007) Expression of an NADP-malic enzyme gene in rice (*Oryza sativa*. L) is induced by environmental stresses; over-expression of the gene in *Arabidopsis* confers salt and osmotic stress tolerance. *Plant Mol Biol*, 64: 49–58. Doi: 10.1007/s11103-007-9133-3

Monteiro-Batista R.C., Siqueira J.A., da Fonseca-Pereira P., Barreto P., Feitosa-Araujo E., Araújo W.L., Nunes-Nesi A. (2025) Potential roles of mitochondrial carrier proteins in plant responses to abiotic stress. *Journal of Experimental Botany*, eraf032. Doi: 10.1093/jxb/eraf032

Nezhadahmadi A., Prodhan Z.H., Faruq G. (2013) Drought tolerance in wheat. *Sci. World J.*, 12 p. Doi: 10.1155/2013/610721

Niyazova N.N., Huseynova I.M. (2024) The antioxidant defense system of tomato (*Solanum lycopersicum* L.) varieties under drought stress and upon post-drought rewatering. *Biochemistry*, 89(6):1146–1157. Doi: 10.1134/S0006297924060130.

Sun X., Han G., Meng Z., Lin L., Sui N. (2019) Roles of malic enzymes in plant development and stress responses. *Plant Signal Behav.*,

14(10):e1644596. Doi: 10.1080/15592324.2019.1644596

Tanaka A., Ito H. (2025) Chlorophyll degradation and its physiological function. *Plant Cell Physiol*, 66(2): 139–152. Doi: 10.1093/pcp/pcae093

Turner N.C. (1981) Techniques and experimental approaches for the measurement of plant water status. *Plant Soil*, 58: 339–366. Doi: 10.1007/BF02180062

Wei H., Lu Z., Jiang H., Xue C., Xu X., Liu G., Lian B., Zhong F., Movahedi A., Zhang J. (2025) Comprehensive analysis of PEPC gene family in *Populus trichocarpa*: characterization, evolutionary insights, and the role of PtPEPC4-PtLTPG14 interaction in carbon metabolism. *Plant Physiol Biochem*, 220:109573. Doi: 10.1016/j.plaphy.2025.109573

Yang M., Geng M., Shen P., Chen X., Li Y., Wen X. (2019): Effect of post-silking drought stress on the expression profiles of genes involved in carbon and nitrogen metabolism during leaf senescence in maize (*Zea mays* L.), *Plant Physiol. Biochem*, 135:304-309. Doi: 10.1016/j.jhazmat.2021.127159

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.

References

Akhoon B.A., Gupta S.K., Dhar M.K. (2023) Dissecting the genome, secretome, and effectome repertoires of *Monilinia* spp.: The causal agent of brown rot disease: A comparative analysis. *Postharvest Biol. Technol*., 195:112120

Boehm E.W.A., Ma Z., Michailides T.J. (2001) Species-specific detection of *Monilinia fructicola* from California stone fruits and flowers. *Phytopathology*, 91:428–439.

Bustin S.A., Beaulieu J.F., Huggett J., Jaggi R., Kibenge F.S.B., Olsvik P.A., Penning L.C., Toegel S. (2010) MIQE précis: Practical implementation of minimum standard guidelines for fluorescence-based quantitative real-time PCR experiments. *BMC Mol. Biol*., 11:74.

Chen F., Liu X., Schnabel G. (2013) Field strains of *Monilinia fructicola* resistant to both MBC and DMI fungicides isolated from stone fruit orchards in the Eastern United States. Plant Dis., 97:1063–1068.

Côté M.J., Tardif M.C., Meldrum A.J. (2004) Identification of *Monilinia fructigena*, *M. fructicola*, *M. laxa*, and *Monilia polystroma* on inoculated and naturally infected fruit using multiplex PCR. *Plant Disease*, 88:1219–1225.

Fischer J.M.M., Savi D.C., Aluizio R., May De Mio L.L., Glienke C. (2017) Characterization of *Monilinia* species associated with brown rot in stone fruit in Brazil. *Plant Pathol.*, 66:423–436.

Förster H., Adaskaveg J.E. (2000) Early brown rot infections in sweet cherry fruit are detected by *Monilinia*-specific DNA primers. *Phytopathology*, 90:171–178.

Hilber-Bodmer M., Knorst V., Smits T. H.M., Patocchi A. (2012) First report of Asian brown rot caused by *Monilia polystroma* on apricot in Switzerland. *Plant Disease*, 96:146.

Hrustic J., Mihajlovic M., Grahovac M., Delibasic G., Bulajic A., Krstic B., Brankica T. (2012) Genus *Monilinia* on pome and stone fruit species. *Pestic. Fitomed*., 27:283–297.

Jia F., Chen M., Liu C., Chen S., Liu W.,

Huang K., Sun X., Ma G., Chen G. (2024) *Streptomyces rapamycinicus* HCD1–10: an effective biocontrol actinomycete against postharvest Chinese flat peach brown rot caused by *Monilinia fructicola*. *Sci. Hortic*., 327:112836

Martini C., Lantos A., Di Francesco A., Guidareli M., D'Aquino S., Baraldi E. (2014) First report of Asiatic brown rot caused by *Monilinia polystroma* on peach in Italy. *Plant Disease*, 98:1585.

Petróczy M., Szigethy A., Palkovics L. (2012) *Monilinia* species in Hungary: Morphology, culture characteristics, and molecular analysis. *Trees* (Berl.), 26:153–164.

Poniatowska A., Michalecka M., Bielenin A. (2013) Characteristic of *Monilinia* spp. Fungi causing brown rot of pome and stone fruits in Poland. *Eur. J. Plant Pathol*., 135:855–865.

Poniatowska A., Michalecka M., Puławska J. (2021) Phylogenetic relationships and genetic diversity of *Monilinia* spp. isolated in Poland based on housekeeping- and pathogenicity-related gene sequence analysis. *Plant Pathol*., 70:1640–1650

Riccioni L., Valente M.T. (2015) Comparison of different PCR tests to identify *Monilinia fructicola*. *Bull. OEPP/EPPO Bull*., 45:33–40.

Van Brouwershaven I.R., Bruil M.L., Van Leeuwen G.C.M., Kox L.F.F. (2010) A real-time (TaqMan) PCR assay to differentiate *Monilinia fructicola* from other brown rot fungi of fruit crops. *Plant Pathol.*, 59:548–555.

Vasić M., Duduk N., Ivanović M.S. (2013) First report of brown rot caused by *Monilia polystroma* on apple in Serbia. *Plant Disease*, 97:145.

BIOLOGICAL CONTROL METHODS AGAINST PLANT DISEASES: MODERN APPROACHES AND APPLICATION PROSPECTS

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Abstract: Plant diseases are among the major factors limiting agricultural productivity and crop quality worldwide. The excessive use of chemical pesticides has resulted in environmental pollution, the development of pathogen resistance, and negative impacts on human health, increasing the demand for environmentally friendly alternatives. The present study evaluated the effectiveness of biological control agents, particularly *Trichoderma harzianum*-based biopesticides, in reducing disease incidence and improving the growth and productivity of tomato plants under greenhouse conditions. Comparative analyses were conducted between control and treated variants by assessing disease incidence, disease severity, vegetative growth parameters, and yield characteristics. The application of *Trichoderma* significantly reduced disease incidence from 48.0% to 18.0% and disease severity from 35.0% to 12.0%. Moreover, treated plants exhibited improvements in plant height, root length, vegetative biomass, fruit number, and overall yield. Yield increased from 4.8 to 6.5 kg m^{-2}, while the proportion of marketable fruits increased from 74% to 90%. The positive effects of the biological preparation were mainly associated with mycoparasitism, competition for nutrients and ecological niches, production of antimicrobial metabolites, and activation of induced systemic resistance in plants. In addition to suppressing soil-borne pathogens such as *Fusarium* spp., *Rhizoctonia solani*, and *Pythium* spp., biological treatments improved soil microbiological activity and reduced dependence on chemical pesticides. The results obtained demonstrate that *Trichoderma*-based biopesticides represent an effective, environmentally safe, and sustainable alternative for disease management in tomato production and support their wider application within integrated plant protection systems.
Keywords: *Trichoderma harzianum*, tomato, biopesticide, induced systemic resistance, sustainable agriculture, integrated pest management.

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Introduction

Plant diseases and their characteristics. The widespread use of chemical fertilizers and pesticides has led to increased productivity in agriculture. However, the long-term use of these substances has had negative effects on the environment, the biological balance of the soil, and human health. Therefore, there has been increased interest in environmentally safe and sustainable control methods in modern agriculture.

Plant diseases are disruptions in the normal physiological function of plants as a result of pathogenic organisms or unfavorable environmental factors. These diseases weaken

plant development, reduce yield, and sometimes lead to plant death.

Plant diseases are mainly divided into three groups:

- **Fungal diseases** are the most widespread group of diseases. It spreads through spores and is characterized by leaf spotting, root, stem and fruit rot.
- **Bacterial diseases** are caused by bacteria entering the plant through wounds or natural openings. The main symptoms are softening of tissues, watery spots and rot.
- **Viral diseases** occur as a result of viruses disrupting the functioning of plant cells. Mosaic symptoms are observed with color changes, impaired development, and fruit deformation.

The spread of diseases is influenced by climatic conditions, soil and water quality, as well as agrotechnical errors. Especially high humidity, improper irrigation, and violation of the crop rotation system accelerate the development of pathogens. Similar pathology is observed in plants due to the influence of several different factors (see Table 1).

Plant diseases cause reduced productivity and economic losses. At the same time, excessive use of chemicals to combat diseases leads to pollution of soil and water resources, a decrease in beneficial microorganisms, and risks to human health. For this reason, the application of biological control methods has become increasingly important in recent years (Cook & Baker 1983; Lahlali et al. 2022).

Causes of the emergence and spread of diseases. The spread of plant diseases occurs as a result of the interaction between the pathogen, the host plant, and environmental factors, and this process is explained by the principle of the "Disease Triangle". The main factors influencing the occurrence and epidemiological dynamics of diseases are:

Table 1. Similar symptoms of infectious and non-infectious diseases

Plant name	Non-infectious disease	Cause of the disease	Infectious disease with similar symptoms
Tomato (*Solanum lycopersicum* L.)	Blossom end-rot	Frequent changes in humidity, lack of calcium in the soil	Bacterial Blossom end-rot (*Bacterium lycopersici*)
Pepper (*Capsicum annuum* L.)	Wilting and chlorosis	Iron deficiency	Stolbur (viral disease)
	Blossom end-rot	Frequent changes in humidity, lack of calcium in the soil	Bacterial Blossom end-rot (*Bacterium lycopersici*)
	Wilting and Root Browning	Low temperature in the soil	Bacterial Blossom end-rot (*Clavibacter michiganensis* subsp. *michiganensis*)

a) Climatic factors: Temperature, humidity, and precipitation play an important role in the development of plant diseases. High humidity and optimal temperature conditions accelerate the spread of fungal and bacterial diseases. Wind and air currents allow spores to be carried over long distances.

b) Soil characteristics: Soil structure, nutrient supply, pH, and drainage characteristics affect plant resistance to disease. Poor drainage and excessive moisture promote the development of root rot and other soil-borne diseases.

c) Irrigation regime: Excessive or improperly organized irrigation causes increased humidity in the area and creates favorable conditions for the development of pathogens. Water droplets that remain on the leaf surface for a long time, in particular, accelerate the spread of infection.

d) Agrotechnical errors: Failure to follow crop rotation, monoculture, dense planting of plants, and use of contaminated tools and equipment increase the risk of disease spread. Pathogens can easily be transported from one area to another as a result of human

activity.

e) Modern monitoring methods: PCR-based molecular diagnostics, Geographic Information Systems (GIS), GPS technologies, and remote sensing methods are widely used for early detection and prediction of the spread of plant diseases. These methods allow for the identification of disease foci and the planning of effective control measures (Agrios 2005).

Economic and ecological impacts of plant diseases. Plant diseases cause reduced productivity in agriculture, deterioration of product quality, and serious economic losses. These losses are divided into two groups: direct and indirect. Direct losses include crop loss in the field, decay during storage, and loss of quality. Indirect losses are related to changes in market prices, shortages of raw materials in the processing industry, and limited export opportunities (FAO 2021).

Plant diseases negatively affect not only the quantity of the crop, but also its quality indicators. As a result of the weakening of photosynthesis, the amount of sugar, vitamins, and dry matter in the crop decreases. In addition, as a result of some fungal diseases, mycotoxins that are dangerous to human health accumulate in the product, which limits the use and sale of the product.

From an ecological perspective, the intensive use of chemicals to combat diseases leads to the pollution of soil and water resources, the decline of beneficial microorganisms, and the weakening of biodiversity. At the same time, the widespread spread of disease-susceptible varieties and the decline of local genetic resources increase the risk of losing important genetic material for future breeding efforts.

Global climate change creates conditions for the spread of new pathogens, which poses an additional threat to the sustainability of native plant species. Crop losses caused by plant diseases can negatively affect the economic situation of small and medium-sized farms, especially in rural areas, and can lead to social problems.

Thus, plant diseases are one of the problems of great importance not only from an agrobiological perspective, but also from an economic, ecological, and social perspective (FAO 2021).

The role and problems of chemical control: Chemical methods have been used as the main means of combating plant diseases and pests for many years. Although these methods are highly effective in the short term, their widespread and continuous application poses serious risks to human health and the environment.

Effects of pesticides on human health: Pesticides are potentially toxic substances and can have both acute and chronic effects on the human body. Acute effects are mainly characterized by poisoning, respiratory problems, and skin diseases. Long-term effects are associated with weakened immune systems, impaired reproductive health, and genetic changes.

Pesticide residues in food products: The use of chemical products can result in pesticide residues on products. In some cases, these residues exceed the maximum limits set, posing a threat to human health. In particular, systemic drugs accumulate in plant tissues and cannot be completely removed by conventional washing processes.

Environmental impacts: Most pesticides are released into the soil, water, and air before reaching the target organism. This leads to a decline in soil microorganisms, a weakening of biodiversity, and a disruption of ecosystem balance.

Resistance problem: Prolonged use of the same chemical preparations leads to pathogens gaining resistance to those substances. As a result, there is a need to use higher doses and new drugs, which further increases the environmental burden.

The importance of applying biological control. The health and environmental risks posed by chemical control have necessitated the development of alternative control methods. In this regard, biological control methods are of particular importance in terms of ensuring ecological safety, reducing pesticide burden, and contributing to the formation of sustainable agriculture (WHO 2023).

The essence, definition and fundamental principles of biological control. Biological control or biocontrol is an environmentally safe method of combating plant diseases and pests based on the use of beneficial living organisms, their metabolites, and microbiological products. The main goal of this method is not only to destroy pathogens, but also to preserve and restore the natural balance in the agroecosystem. In modern agriculture, biological control is considered one of the key components of sustainable production and serves to reduce dependence on chemical pesticides.

The basis of biological control is competition between microorganisms for nutrients and habitat, antagonistic effects, and natural regulatory mechanisms. As a result of the activity of beneficial microorganisms, unfavorable conditions are created for the development of pathogens, and the risk of infection is reduced.

The scientific nature and impact of biocontrol. The scientific basis of biological control is based on research conducted in the fields of microbiology, phytopathology, ecology, and molecular biology. Biocontrol agents settle in the rhizosphere and phyllosphere of the plant, creating a protective microbiological environment and limiting the development of pathogens in those areas.

Unlike chemical pesticides, biological agents increase the microbiological activity of the soil, accelerate the circulation of nutrients, and improve the overall physiological condition of the plant. In addition, some biocontrol microorganisms have a positive effect on increasing productivity by increasing the synthesis of hormone-like substances that stimulate plant growth.

Fundamental principles. The effectiveness of biological control is based on the following main mechanisms:

- **Antagonistic impact** – beneficial microorganisms inhibit the development of pathogens by synthesizing antibiotics, enzymes, and other metabolites.
- **Competition for food and habitat –** beneficial microorganisms limit the development of pathogens by utilizing nutrients more quickly.
- **Induced systemic resistance (ISR)** – biological agents activate the plant's defense mechanisms, increasing its resistance to diseases.
- **Siderophore production –** some bacteria synthesize substances that bind iron ions, reducing the availability of elements essential for the development of pathogens.
- **Hyperparasitism –** some microorganisms grow on pathogens and directly destroy them. This feature is especially characteristic of Trichoderma species (Compant et al. 2010).

Methods of applying biological control. The effectiveness of biological control depends on the method of application of the biological agents used. The main application methods are as follows:

- **Inoculation method –** the introduction of a small amount of a biological agent into the soil or plant and subsequent formation of a protective effect through natural reproduction.
- **Augmentation method** – application of biological preparations multiplied in the laboratory to a large area.
- **Seed biopreparation** – the treatment of seeds with beneficial microorganisms before sowing and the creation of an initial protective microflora.
- **Soil treatment method** - application of biological preparations through an irrigation system or directly to the soil.
- **Foliar spraying –** the application of biological preparations to protect the aboveground organs of the plant from diseases.

The correct selection and combination of these methods increase the effectiveness of biological control and allows for the formation of a sustainable plant protection system (Glick 2012).

Ecological and economic perspectives. Biological control plays an important role in the development of environmentally safe and sustainable agriculture. While intensive use of chemical pesticides leads to a decrease in soil

biological activity and environmental pollution, biological methods help preserve soil fertility and biodiversity. In addition, reducing pesticide residues serves to protect human health and ensure food security. From an economic perspective, although the application of biological preparations requires additional costs at the initial stage, in the long term, economic efficiency is ensured by reducing pesticide consumption, minimizing crop losses, and preserving soil fertility. For this reason, biological control is considered one of the promising and sustainable directions of modern agriculture (Du et al. 2022).

Taxonomic and functional characteristics of biological control agents. Biological Control Agents (BCAs) are beneficial microorganisms that limit the development of plant disease pathogens and increase the defenses of plants. These agents include bacteria, fungi, actinomycetes, and other microorganisms, and act through mechanisms such as antagonism, antibiosis, competition, and induction of systemic resistance.

Molecular and biological potential of bacterial agents. Bacteria are one of the most widely used groups of microorganisms in biological control. They mainly inhabit the rhizosphere and phyllosphere, limiting the development of pathogens and stimulating plant growth.

Characteristics of the genus *Bacillus*. *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Bacillus pumilus* species are widely used in the preparation of biological preparations because they produce highly resistant spores (Fig. 1). *B. amyloliquefaciens* is one of the most common bacteria that colonize plants endophytically and plays an important role in the biocontrol of vascular plant pathogens (Choudhary & Johri 2009). These bacteria damage the cell wall and membrane of pathogens by synthesizing enzymes such as chitinase, β-glucanase, and protease, as well as antimicrobial substances such as iturin, surfactin, and fengycin. *Bacillus* species are particularly effective against pathogens belonging to the genera *Fusarium*, *Phytophthora*, *Alternaria* and *Rhizoctonia*, and are also involved in the synthesis of substances that stimulate plant growth.

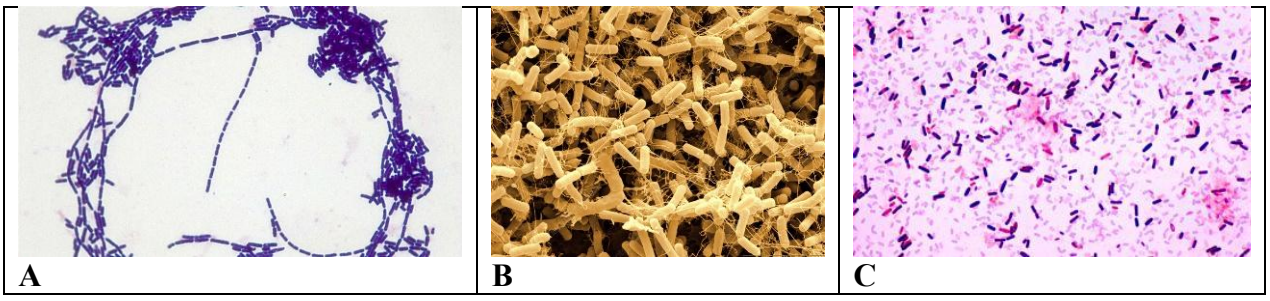

Figure 1. *Bacillus* genus bacteria: a) *B. subtilis* b) *B. amyloliquefaciens* c) *B. pumilus*.
Source: https://pmc.ncbi.nlm.nih.gov/articles/PMC10536829/

Functional characteristics of the genus Pseudomonas: Species such as *Pseudomonas fluorescens* form biofilms on the root surface, preventing the colonization of pathogens (Fig. 2). These bacteria bind iron ions through siderophores and synthesize various antibiotic substances and antimicrobial metabolites, limiting the development of pathogens. In addition, *Pseudomonas* species activate induced systemic resistance in plants, increasing their resistance to diseases and environmental stresses.

Mycoparasitic activity of antagonistic fungi. Among biological agents of fungal origin, the most widely used genus is *Trichoderma*. Characteristics of the *Trichoderma* genus: *Trichoderma harzianum, T. viride,* and *T. asperellum* are fungi that are widely distributed in the soil and have high antagonistic activity. These species limit the access of pathogens to food sources and disrupt their hyphae through the mechanism of

mycoparasitism. The chitinase, β-glucanase, and protease enzymes synthesized by *Trichoderma* destroy the cell wall of pathogens, resulting in their growth being halted. These fungi are particularly effective against soil-borne pathogens such as *Fusarium* spp., *Sclerotinia* spp., *Pythium* spp., and *Rhizoctonia solani*. In addition, *Trichoderma* species activate systemic resistance in plants, improve nutrient uptake, and have a positive effect on plant development.

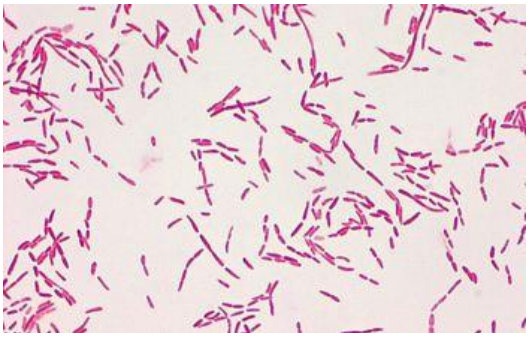

Figure 2. *Pseudomonas fluorescens.*

Actinomycetes and antibiotic production: Among actinomycetes, the most important biological control agents are species belonging to the genus *Streptomyces*. These microorganisms limit the development of pathogens by synthesizing streptomycin, neomycin, and other antibiotic substances. *Streptomyces* species participate in the decomposition of organic matter, improve soil structure, and accelerate humus formation. In addition, they synthesize various enzymes and growth stimulants, which have a positive effect on plant development and increase their resistance to diseases.

Bacteriophages: specific biological defenses. Bacteriophages are viruses that infect only bacteria and have high specificity against phytopathogenic bacteria. They target only certain bacterial pathogens without affecting beneficial microorganisms, making them an environmentally safe biological control tool. After bacteriophages enter a bacterial cell, they multiply inside it, eventually causing the cell to lyse. This mechanism allows the pathogen population to decrease in a short time. *Bacteriophages* are mainly used against bacterial pathogens such as *Erwinia amylovora, Xanthomonas* spp., *Pseudomonas syringae*, and *Ralstonia solanacearum*. The development of molecular biology and genomic technologies has expanded the application possibilities of bacteriophages in biological control. Their high specificity and environmental safety increase their importance in modern plant protection.

Mechanisms of action of biological control agents. Biological control agents limit the development of pathogens through various biological and biochemical mechanisms. In most cases, several mechanisms of action act simultaneously, weakening the development of diseases and increasing the defenses of plants. The main mechanisms of action include antibiosis, competition, parasitism, and induced systemic resistance.

Antibiosis mechanism.: During antibiosis, antibiotics and other biologically active substances synthesized by beneficial microorganisms weaken or completely stop the development of pathogens. Iturin, surfactin, and fengycin synthesized by *Bacillus* species, as well as phenazines and other metabolites produced by Pseudomonas species, disrupt the cellular structure of pathogens, leading to their destruction.

Competition mechanism: Beneficial microorganisms compete with pathogens for nutrients and living space. They rapidly colonize plant roots and leaf surfaces, making it difficult for pathogens to settle. Reduced availability of iron ions as a result of siderophore production also limits the development of pathogens.

Parasitism and mycoparasitism: During parasitism, biological agents grow directly on

the pathogenic organism and destroy it. This mechanism is particularly characteristic of *Trichoderma* species. In the process of mycoparasitism, enzymes such as chitinase, β-glucanase, and protease break down the cell wall of pathogenic fungi and stop their vital activity. This mechanism of action is highly effective against pathogens such as *Fusarium* spp., *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Pythium* spp., and *Botrytis cinerea*. The activity of bacteriophages against bacterial pathogens is also considered a special form of parasitism mechanism (Haas & Défago 2005).

Induced systemic resistance: Some biological control agents activate the defense system of plants, increasing their resistance to diseases. This process is called induced systemic resistance (ISR). As a result of ISR, the synthesis of defense proteins and antioxidant enzymes is enhanced, the plant's response to infections accelerated, and the level of disease development is reduced. Thus, the multifaceted mechanisms of action of biological control agents ensure their high effectiveness in plant protection and play an important role in the development of sustainable agriculture.

Induced systemic resistance. Induced systemic resistance (ISR) is one of the most important mechanisms of biological control and is formed as a result of the interaction between beneficial microorganisms and plants. Signal molecules secreted by beneficial bacteria and fungi activate the plant's defense system, enhancing the synthesis of various protective proteins, phytoalexins, and antioxidant enzymes. As a result, the plant becomes more resistant to pathogen attacks, disease development is weakened, and crop losses are reduced. It has been determined that *Bacillus subtilis, Pseudomonas fluorescens,* and *Trichoderma harzianum* species play an important role in the formation of the ISR mechanism. Induced systemic resistance increases the resilience of plants not only to biotic stress factors, but also to abiotic stress factors such as drought, salinity, and temperature changes. This feature demonstrates the importance of biological control agents in terms of both plant protection and productivity enhancement (Köhl et al. 2019).

Biopesticides and phytobiological preparations. Biopesticides are preparations based on microorganisms, their metabolites, plant extracts, and other biologically active substances. These preparations are used to limit the development of pathogens, pests, and weeds. Unlike chemical pesticides, biopesticides do not accumulate in the environment, do not disrupt the microbiological balance of the soil, and ensure environmental safety.

Biopesticides of microbiological origin. Microbiological biopesticides are mainly made from bacteria, fungi, and viruses and are widely used in modern agriculture (Figure 3). Biopesticides are effective tools for controlling insects, pathogens, and weeds. However, biopesticides require more knowledge about application and the biology of target pests compared to broad-spectrum pesticides. Biopesticides are safer for human health and the environment, but they usually take longer to kill pests than traditional insecticides. Therefore, pest species may be observed for several days after initial biopesticide applications. Compared to traditional insecticides, biopesticides typically persist in the environment for a shorter period of time and therefore have fewer trade barriers. However, this may require more frequent application.

- Preparations based on *Bacillus subtilis* and *B. amyloliquefaciens* inhibit the development of phytopathogens by synthesizing antibiotic substances, lipopeptides, and hydrolytic enzymes. These drugs are especially effective against fungal diseases.
- *Trichoderma harzianum* based preparations inhibit the development of soil-borne pathogens such as *Fusarium*, *Rhizoctonia*, *Sclerotinia*, and *Pythium* through mechanisms of mycoparasitism and antagonism (Fig. 4).
- *Pseudomonas fluorescens* preparations limit the development of pathogens through siderophore production and synthesis of antibiotic metabolites, while also improving the development of the

plant root system and the absorption of nutrients.

- *Bacillus thuringiensis* (Bt)-based preparations are mainly used against insect pests. The crystal proteins synthesized by this bacterium affect the digestive system of insects, causing their destruction. The fact that these preparations are safe for humans, animals, and beneficial insects allows for their widespread use (Fravel 2005).

Thus, biopesticides of microbial origin, in addition to being an environmentally safe alternative to chemical preparations, play an important role in the development of sustainable agricultural systems.

Phytobiological preparations and plant extracts. Plant-based biological preparations occupy an important place among natural defenses. Biologically active substances synthesized by various plants have a protective effect against pathogens and pests, and extracts obtained from these substances are used as biopreparations in agriculture.

Garlic extract has a strong antibacterial and antifungal effect due to the allicin it contains. Hot pepper extract limits the development of pests through capsaicin. Nettle extract not only improves plant nutrition but also increases their resistance to disease. Tobacco and chamomile extracts have insecticidal and antimicrobial properties.

In recent years, the use of preparations based on essential oils from plants such as mint, thyme, eucalyptus, rosemary, and tea tree has expanded. The terpenoids and phenolic compounds contained in these oils damage the cell membranes of pathogens, limiting their development. Therefore, plant extracts and essential oils are of great importance in the preparation of environmentally safe biological preparations.

Figure 3. Biopesticides.

Advantages and application prospects of biopesticides. One of the main advantages of biopesticides is their easy integration into integrated crop protection systems. These preparations allow you to reduce the use of chemical pesticides, maintain the microbiological balance of the soil, and prevent the formation of pesticide residues in products. Additionally, they are considered safer for beneficial insects and pollinators.

The effectiveness of biopesticides depends on factors such as temperature, humidity, soil pH, and application method. Therefore, the correct implementation of agrotechnical measures during their use is of particular importance. Currently, the biopesticide industry is rapidly developing worldwide, and numerous bacterial, fungal, and plant-based preparations are being produced commercially. Advances in biotechnology and molecular biology have enabled the creation of new generation of biological preparations, and their role in agriculture is expected to increase in the future (Verma et al. 2024).

Figure 4. *Rhizoctonia*.
Source: https://vegpath.plantpath.wisc.edu/diseases/rhizoctonia/

Application and advantages of biopesticides based on microorganisms. Biopesticides based on microorganisms are one of the most promising areas of modern plant protection. These preparations are made from living cells, spores, or their metabolites of bacteria, fungi, viruses, and other beneficial microorganisms. In addition to controlling plant diseases and pests, microbiological preparations also serve to preserve the biological activity of the soil and ensure the stability of agroecosystems. Their natural origin supports the activity of beneficial microorganisms, activates biological processes in the soil, and increases the resistance of plants to various biotic and abiotic stress factors. As a result of the development of biotechnology, it has become possible to prepare a new generation of highly effective microbiological preparations. These preparations have great promise in terms of ensuring environmental safety, reducing pesticide burden, and contributing to the development of sustainable agriculture.

Application of bacterial biopesticides. Among bacterial biopesticides, the species *Bacillus subtilis, B. amyloliquefaciens,* and *B. pumilus* are of particular importance. These bacteria limit the development of phytopathogens by synthesizing enzymes, antibiotic substances, and lipopeptides, while simultaneously activating induced systemic resistance in plants. They are applied by seed treatment, soil bio enrichment, and foliar spray methods.

Application of fungal biopesticides. Among mushroom-based biopreparations, *Trichoderma harzianum, T. viride,* and *T. asperellum* species are widely used. These fungi inhibit the development of pathogens through mycoparasitism, enzymatic activity, and competition for nutrients. They are especially effective against soil-borne diseases such as *Fusarium*, *Rhizoctonia*, *Pythium* and *Sclerotinia*.

Viral biopesticides. The main representatives of virus-based biopreparations are baculoviruses. They are highly specific in that they only infect certain insect pests and do not pose a threat to beneficial organisms. This feature makes them an environmentally safe means of control.

Effects of microorganism-based biopesticides. Biopesticides act through mechanisms of antibiosis, competition, parasitism, mycoparasitism, and induced systemic resistance. As a result of these processes, the population of pathogens decreases and the resistance of plants to diseases increases.

Environmental and economic advantages of biopesticides. Biological preparations do not create toxic residues in the environment, protect soil microflora, and help maintain biodiversity. Their application reduces pesticide residues in products, reduces dependence on chemicals, and helps reduce production costs.

Industrial production and prospects of biopesticides. As a result of the development of biotechnology, the industrial production of bacterial, fungal, and viral biopesticides has expanded. Although the effectiveness of these preparations depends on environmental factors, their role in sustainable agriculture is increasing and is expected to be more widely used in the

future (Verma et al. 2024).

Modern directions and prospects of biological control of plant diseases. The intensive use of chemical pesticides against plant diseases has led to problems such as environmental pollution, the decline of beneficial organisms, and the emergence of resistance in pathogens. For this reason, biological control has gained widespread importance as an environmentally safe and sustainable alternative in modern agriculture. As a result of the development of biotechnology and microbiology, highly effective biopreparations have been developed based on microorganisms belonging to the genera *Bacillus*, *Pseudomonas*, and *Trichoderma*. These microorganisms not only limit the development of phytopathogens but also increase the absorption of nutrients and productivity of plants. The combined use of several beneficial microorganisms further enhances their effectiveness.

The study of the plant microbiome has opened new perspectives in biological control. The selection and application of beneficial microorganisms allow for the creation of more effective and sustainable biopreparations. At the same time, molecular biology and genomic technologies are enabling the identification of new strains with high activity.

Modern production technologies and microencapsulation methods have increased the shelf life of biopreparations and their resistance to environmental factors. In addition, the application of biological agents in integrated plant protection systems, together with agrotechnical measures and resistant varieties, provides high efficiency.

In recent years, the development of nanobiotechnology has created new opportunities to increase the duration of action of biological preparations and more effectively deliver active ingredients to plant tissues. The growing interest in ecologically clean production in the world and in Azerbaijan has led to the expansion of the application of biological control agents.

Thus, biological control will be one of the main directions of sustainable agriculture in the future, playing an important role in increasing productivity, managing diseases, and protecting agroecosystems (He et al. 2021).

Materials and Methods

Research area and conditions. The research was conducted on tomato plants in greenhouse conditions. Since the greenhouse environment allows for control of factors such as temperature, humidity, lighting, and irrigation, it has created favorable conditions for evaluating the effectiveness of biological control agents. Vegetable plants were selected as the object of the study, and they were cultivated according to standard agrotechnical rules. During the growing season, plants were watered, fed, and ventilated regularly, and special attention was paid to maintaining constant temperature and humidity levels within the greenhouse.

Figure 5. Grey leaf spot on tomato.

The randomization principle was applied when placing experimental variants, minimizing the impact of microclimate differences within the greenhouse on the results. The physical and biological properties of the substrate where the plants are grown were considered, and the stability of soil conditions was ensured. During the observations, plant height, leaf number, vegetative development indicators, prevalence of disease symptoms, and general phytosanitary condition were recorded during the observations. Changes observed in the leaf, stem, and root system were evaluated separately. During the research, sanitary and phytosanitary regulations were followed, the tools used were disinfected, and diseased plant residues were removed from the field in a timely manner. However, in some cases, several diseases are observed in tomato plants (Figs. 5, 6). Research conducted in greenhouse conditions allows for an accurate assessment of the effects of biological control agents and a scientific analysis of the results obtained.

Figure 6. Tomato internal blossom end-rot (IBER).

Applied biological agents. During the study, various biological agents were used to combat plant diseases (Table 2). Biological agents are distinguished by their environmental safety, their lack of toxic residues in crops, and their reduced risk of pathogen resistance. During the study, a comparative analysis was conducted between the control and study groups using the *Trichoderma* biopesticide. Microorganisms belonging to the genera *Bacillus*, *Pseudomonas*, and *Trichoderma* were used as the main biological agents in the study. *Bacillus* species have been shown to inhibit the growth of phytopathogens by synthesizing antibiotic substances, enzymes, and lipopeptides, while simultaneously stimulating plant nutrient uptake and growth (Lugtenberg & Kamilova 2009).

Bacteria of the genus *Pseudomonas* formed beneficial microflora in the root zone, competing with pathogens for nutrients, and weakened the development of pathogens through the synthesis of siderophores and other metabolites. In addition, these bacteria have contributed to the activation of systemic resistance mechanisms in plants. Fungi belonging to the genus *Trichoderma* has been used against soil-borne diseases. These microorganisms limited the growth of pathogenic fungi by breaking down their hyphae and improved the development of the root system and the absorption of nutrients. The effectiveness of biological agents has depended on their proper selection, timing of application, and environmental conditions. Therefore, the preparations were applied under optimal temperature and humidity conditions, and agrotechnical measures were fully observed. Thus, applied biological agents have made a significant contribution to reducing the spread of diseases, improving plant development, and producing environmentally safe products

(Villavicencio-Vásquez 2025).

Research method. Studies were conducted in greenhouse conditions during the growing season to assess the effectiveness of biological control agents against plant diseases. The experiments were organized under the same agrotechnical conditions, the difference between the variants was only related to the biological agents applied. In the control variant, biological preparations were not used, while in the experimental variant, biological preparations prepared based on beneficial microorganisms were applied in accordance with the manufacturer's recommendations in the norms and periods. When applying powder or granule preparations, they are first dissolved in a small amount of water, then water is added to the required volume to prepare a homogeneous solution. Usually, *Trichoderma* preparations are prepared at a rate of 2–5 g of the preparation per one liter of water or according to the manufacturer's recommended dosage. The prepared solution should be used immediately without prolonged storage to preserve the viability and activity of microorganisms.

Table 2. Areas of application of biological control agents.

Biological control agent	Active agent	Target disease or pathogen	Mechanism of action	Effect observed on tomato plants
Bacterial	*Bacillus subtilis*	*Fusarium oxysporum*, *Alternaria solani*, bacterial spot	Antibiosis, competition, induction of ISR	Disease spread is reduced; root development and vegetative growth are improved
Bacterial	*Bacillus amyloliquefaciens*	Root rot; leaf spots	Antibiotic and lipopeptide synthesis	Plant height and yield increase, disease pressure decreases
Rhizosphere bacteria	*Pseudomonas fluorescens*	Wilting diseases; root pathogens	Siderophore synthesis, nutrient competition, ISR	The root system is strengthened; nutrient absorption improves
Fungal-based	*Trichoderma harzianum*	*Fusarium* spp.; *Rhizoctonia solani*; *Pythium* spp.	Mycoparasitism, enzyme synthesis, competition	Reduction of root rot and acceleration of vegetative development are observed
Fungal-based	*Trichoderma viride*	Soil-borne fungal diseases	Cell wall breakdown and hyperparasitism	Plant viability and crop quality increase
Plant extract	Garlic extract (*Allium sativum*)	Fungal and bacterial diseases	Antimicrobial effects of allicin	The development of leaf spots and rots is weakened
Plant extract	Nettle extract (*Urtica dioica*)	For preventive protection purposes	Stimulation of plant immunity	Increases stress resistance and vegetative development of plants
Essential oils	Thyme and tea tree oils	Fungal diseases	Cell membrane damage	Pathogen development is limited
Viral-based	Baculovirus	Insect pests	Reproduction in the cells of the pest	The number of pests decreases; crop losses are minimized

In tomato plants, *Trichoderma*-based biopesticides have been used as seed treatment, soil or substrate treatment, root system treatment, application during the vegetation period, and, if necessary, leaf surface spraying. During the observations, plant growth and development indicators, including plant height, leaf number, phytosanitary condition, and disease symptom development intensity, were assessed. The prevalence of diseases was determined as a percentage based on the ratio of infected plants to the total number of plants. Productivity and product quality indicators were also analyzed to assess the effect of biological preparations. The results obtained showed that biological agents reduce the development of diseases and increase the healthy development and productivity of plants. These results confirmed that biological control methods are a promising approach for environmentally safe

and sustainable agriculture (Tariq et al. 2020).

Results and Discussions

During the conducted studies, the effect of biological control agents in preventing plant diseases and increasing productivity was evaluated. During the experiment, observations were made on the control and experimental variants, and the rate of disease development, vegetative development of plants, and yield indicators were compared. As a result of the application of *Trichoderma harzianum*-based biopesticide, the development of soil-borne pathogens, especially *Fusarium* spp., *Rhizoctonia solani* and *Pythium* spp., was significantly reduced in tomato plants. At the same time, positive changes were observed in root system development, vegetative growth and yield indicators. The effect of the preparation is mainly associated with mycoparasitism, competition for nutrients and activation of induced systemic resistance in plants.

Based on the results of the observations, it was determined that the intensity of disease spread in the variants where biological preparations were applied was lower than in the control variant. In particular, the use of preparations based on *Trichoderma* spp. and *Bacillus* spp. has weakened the development of pathogenic microorganisms in the soil and on the surface of plants, and the rate of reproduction of pathogens has decreased. The competitive ability, synthesis of antibiotic substances, and hyperparasitism properties of beneficial microorganisms have played an important role in limiting the development of diseases (Harman et al. 2004).

Table 3. Application of *Trichoderma*-based biopesticide on tomato plants.

Indicator	Control variant	*Trichoderma* applied variant	Change (%)
Disease prevalence (%)	48,0	18,0	−62,5
Disease development rate (%)	35,0	12,0	−65,7
Plant height (cm)	62,4	74,8	+19,9
Number of leaves (pcs/plant)	21	28	+33,3
Root length (cm)	14,5	19,2	+32,4
Vegetative mass (g/plant)	285	358	+25,6
Number of fruits (pcs/plant)	18	24	+33,3
Average fruit weight (g)	82,0	91,5	+11,6
Yield (kg/m²)	4,8	6,5	+35,4
Share of marketable product (%)	74	90	+21,6

During the study, positive changes were also observed in the morphological indicators of the plants. In plants treated with biological preparations, the development of the root system was better, the size of the leaf surface increased, and vegetative development was more intensive. Better development of the root system improved the absorption of nutrients and water, which had a positive effect on the overall physiological condition of the plants. Observations have shown that resistance to stress factors has also increased in plants treated with biological agents. Even in the face of temperature changes, humidity fluctuations, and other unfavorable environmental conditions, plant development has become more stable. This feature is explained by the fact that biological agents activate induced resistance mechanisms in plants. This feature is explained by the fact that biological agents activate induced resistance mechanisms in plants. Fruit formation has become more intensive, and product quality indicators have increased. Crop losses due to diseases have decreased, and the amount of marketable produce has increased. The appearance, color, and storage capacity of the fruits have improved, which has contributed to an increase in the market value of the product. Experiments have shown that the application of biological preparations also has a positive effect on the microbiological activity of the soil. The increase in the number of beneficial microorganisms has accelerated the decomposition of organic matter in the soil and

improved the absorption of nutrients by plants. As a result, soil fertility has been preserved, and the biological balance of the agroecosystem has been maintained. The application of biological control methods has also allowed for a reduction in the use of chemical pesticides. This has reduced the risk of environmental pollution and prevented the accumulation of pesticide residues in soil and crops. Biological preparations are considered more favorable in terms of safety for human health and beneficial organisms. At the same time, minimizing the negative impact on soil microflora is of great importance for the formation of sustainable agricultural systems. During observations, it was determined that the effectiveness of biological control agents can vary depending on environmental conditions, temperature, humidity, soil properties, and application technology. However, when used under optimal conditions, biological drugs demonstrate high efficacy and provide significant results in disease management. The results obtained show that the application of biological control methods has a positive effect on reducing the spread of plant diseases, increasing productivity and product quality, protecting soil fertility, and ensuring ecological safety. Therefore, the widespread use of biological preparations in agriculture is considered one of the promising directions in terms of the development of sustainable production systems (Yao et al. 2023).

The results of the conducted studies have shown that the application of biological control agents is highly effective in controlling plant diseases, and the results obtained are consistent with the data presented in international scientific literature. Preparations based on *Trichoderma* spp. and *Bacillus* spp. limited the development of phytopathogens, while simultaneously increasing the vegetative development and productivity of plants (Harman et al. 2004).

The high efficiency of biological preparations is due to their ability to compete with pathogens, synthesize antimicrobial substances, and activate induced systemic resistance in plants. The development of the root system, increase in vegetative mass, and increased resistance to stress factors observed during the study were consistent with the results of other authors (Vinale et al. 2008).

However, the effectiveness of biological agents may vary depending on temperature, humidity, soil properties, and application technology. However, when applied under optimal conditions, they act as an environmentally safe and sustainable alternative to chemical pesticides. The results obtained indicate the feasibility of widespread use of biological preparations in integrated plant protection systems and the importance of directing future research towards the development of new microbiological preparations (Saldaña-Mendoza et al. 2023).

Conclusion

The results of the present study confirmed that biological control agents can effectively reduce the incidence and severity of plant diseases while simultaneously improving plant growth and productivity. The application of *Trichoderma harzianum*-based biopesticides significantly suppressed the development of important soil-borne pathogens and contributed to improved vegetative and reproductive performance in tomato plants.

Compared with the control treatment, plants receiving biological applications showed lower disease prevalence, enhanced root development, increased vegetative biomass, and higher yield and fruit quality. These beneficial effects are primarily associated with the mechanisms of mycoparasitism, competition for nutrients, production of antimicrobial compounds, and induction of systemic resistance in plants. The study further demonstrated that biological preparations positively influence soil microbial activity and contribute to maintaining agroecosystem stability while reducing the need for chemical pesticides and minimizing environmental contamination.

Overall, *Trichoderma*-based biopesticides can be considered a promising component of sustainable agriculture and integrated plant protection systems. Future studies should focus on the development of improved microbial

formulations, optimization of application technologies, and evaluation of their effectiveness under different environmental conditions and cropping systems.

Conflict of Interest

Authors declare that there is no conflict of interest.

References

Agrios G.N. (2005) Plant disease epidemiology. In: Plant Pathology (Fifth Edition). Elsevier.

Choudhary D.K.; Johri B.N. (2009) Interactions of *Bacillus* spp. and plants—with special reference to induced systemic resistance (ISR). *Microbiol. Res.*, 164:493–513.

Compant S., Clément C., Sessitsch A. (2010) Plant growth-promoting bacteria in the rhizosphere and endosphere of plants. Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry*, 42(5): 669–678. Doi: 10.1016/j.soilbio.2009.11.024

Cook R.J., Baker K.F. (1983) The nature and practice of biological control of plant pathogens. APS Press, St.Paul, Minnesota.

Du Y., Wang T., Jiang J., Wang Y., Lv C., Sun K., Sun J., Yan B., Kang C., Guo L., Huang L. (2022) Biological control and plant growth promotion properties of *Streptomyces albidoflavus* St-220 isolated from *Salvia miltiorrhiza* rhizosphere. *Front. Plant Sci*., 13:976813. Doi: 10.3389/fpls.2022.976813

FAO (2021) IPPC Secretariat. Scientific Review of the Impact of Climate Change on Plant Pests.

Fravel D.R. (2005) Commercialization and implementation of biocontrol. *Annual Review of Phytopathology*, 43:337–359. Doi: 10.1146/annurev.phyto.43.032904.09292

Glick B.R. (2012) Plant growth-promoting bacteria: mechanisms and applications. *Scientifica*, 963401.

Haas D., Défago G. (2005) Biological control of soil-borne pathogens by fluorescent *Pseudomonads*. *Nature Reviews Microbiology*, 3(4):307–319. Doi: 10.1038/nrmicro1129.

Harman G.E., Howell C.R., Viterbo A., Chet I., Lorito M. (2004) *Trichoderma* species – opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*, 2(1):43–56. Doi: 10.1038/nrmicro797

He D.C., He M.H., Amalin D.M., Liu W., Alvindia D.G., Zhan J. (2021) Biological control of plant diseases: an evolutionary and eco-economic consideration. *Pathogens*, 10:1311. Doi: 10.3390/pathogens10101311

Köhl J., Kolnaar R., Ravensberg W.J. (2019) Mode of action of microbial biological control agents against plant diseases: Relevance beyond efficacy. *Frontiers in Plant Science*, 10:845. Doi: 10.3389/fpls.2019.00845

Lahlali R., Ezrari S., Radouane N., Kenfaoui J., Esmaeel Q., El Hamss H., Belabess Z., Barka E.A. (2022) Biological control of plant pathogens: a global perspective. *Microorganisms*, 10:596. Doi: 10.3390/microorganisms10030596

Lugtenberg B., Kamilova F. (2009) Plant-growth-promoting *Rhizobacteria*. *Annual Review of Microbiology*, 63:541–556. Doi: 10.1146/annurev.micro.62.081307.162918

Tariq M., Khan A., Asif M., Khan F., Ansari T., Shariq M., Siddiqui M.A. (2020) Biological control: a sustainable and practical approach for plant disease management. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*, 70(6):507–524. Doi: 10.1080/09064710.2020.1784262

Saldaña-Mendoza S.A., Pacios-Michelena S., Palacios-Ponce A.S., Chávez-González M.L., Aguilar C.N. (2023) *Trichoderma* as a biological control agent: mechanisms of action, benefits for crops and development of formulations. *World Journal of Microbiology and Biotechnology*, 3;39(10):269. Doi: 10.1007/s11274-023-03695-0

Verma M.L., Kumar A., Chintagunta A.D., Prashant J.K.S., Bardin M., Lichtfouse E. (2024) Microbial production of biopesticides for sustainable agriculture. *Sustainability*, 16:7496. Doi: 10.3390/su16177496.

Villavicencio-Vásquez M., Espinoza-Lozano F., Espinoza-Lozano L., Coronel-León J. (2025) Biological control agents: mechanisms of action, selection, formulation and challenges in agriculture. *Frontiers in Agronomy*, 7:1578915. Doi: 10.3389/fagro.2025.1578915

Vinale F., Sivasithamparam K., Ghisalberti E.L., Marra R., Woo S.L., Lorito M. (2008) *Trichoderma*–plant–pathogen interactions. *Soil Biology and Biochemistry*, 40: 1–10. Doi: 10.1016/j.soilbio.2007.07.002

World Health Organization (2023) Pesticide Residues in Food. Geneva: WHO, 2023.

Yao X., Guo H., Zhang K., Zhao M., Ruan J., Chen J. (2023) *Trichoderma* and its role in biological control of plant fungal and nematode disease. *Frontiers in Microbiology*, 14:1160551. Doi: 10.3389/fmicb.2023.1160551

MOLECULAR MARKER-BASED STUDY OF THE GENE FOR RESISTANCE TO MILDEW IN SOME AZERBAIJANI TABLE GRAPE VARIETIES

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Abstract: Downy mildew, caused by *Plasmopara viticola*, is one of the most destructive diseases affecting grapevine (*Vitis vinifera* L.), leading to significant yield and quality losses worldwide, including in Azerbaijan. The development and identification of resistant grape cultivars is considered an effective and environmentally sustainable approach to disease management. This study focuses on the molecular marker-based investigation of resistance genes associated with downy mildew in selected table grape varieties cultivated in Azerbaijan. Molecular markers linked to resistance loci were employed to evaluate the genetic potential of different grapevine genotypes. DNA was extracted from young leaf tissues, and specific primers associated with known resistance genes were used in PCR analysis. The presence or absence of target alleles was determined and compared among varieties to assess their resistance profiles. The results demonstrated genetic variability among the studied cultivars in terms of resistance to *Plasmopara viticola*. Certain varieties showed the presence of resistance-associated alleles, indicating their potential use in breeding programs aimed at developing disease-resistant grapevine cultivars. Overall, the application of molecular markers proved to be a reliable and efficient method for early detection of resistance traits, contributing to sustainable viticulture and reduced reliance on chemical control measures.

Keywords: *Vitis vinifera*, *Plasmopara viticola*, downy mildew resistance, molecular marker-assisted selection (MAS), PCR-based genotyping.

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Introduction

Vitis vinifera L. is among the most widely cultivated fruit species worldwide and represents the only *Vitis* species extensively used in winemaking (Reynolds 2015). Viticulture covers around 7.6 million hectares globally, producing more than 67 million tons of grapes annually (Toffolatti et al. 2018b). Despite its agricultural importance, *V. vinifera* is highly susceptible to *Plasmopara viticola* (Berk. et Curt.) Berl. & de Toni, the oomycete causing downy mildew, which can severely reduce grape yield and quality in the absence of effective disease management strategies (Fontaine et al. 2013; Maddalena et al. 2020). The pathogen was introduced to Europe from North America in the nineteenth century and quickly spread, forming genetically distinct populations influenced by geographic and climatic factors (Fontaine et al. 2013; Maddalena et al. 2020). Resistance to downy mildew is naturally found in several non-vinifera North American species such as *Vitis riparia*, *V. cinerea*, and *V. labrusca*, as well as in Northeast Asian species (*Vitis amurensis*), resulting from long-term co-evolution with the pathogen (Jürges et al. 2009). Multiple

quantitative trait loci (QTLs) conferring varying levels of resistance, including *Rpv1*, *Rpv2*, *Rpv3*, *Rpv4*, *Rpv7*, and others, have been identified across these species (Blasi et al. 2011; Merdinoglu et al. 2003; Welter et al. 2007). The exploitation of these resistance sources provides the most sustainable approach to reducing chemical use in viticulture and to manage downy mildew effectively. Traditional management of downy mildew in *V. vinifera* varieties requires frequent fungicide application, which constitutes nearly 70% of agrochemical use in European viticulture. However, overreliance on chemicals leads to environmental pollution, health risks, high costs, and potential development of fungicide-resistant pathogen strains (Hollomon 2015). Therefore, genetic approaches such as marker-assisted selection (MAS) are increasingly adopted to accelerate breeding programs (Eibach & Töpfer 2015). MAS allows the early selection of progeny carrying resistance loci, significantly reducing the time required for cultivar development (Eibach & Töpfer 2015).

Recent studies have reported unique resistance traits in *V. vinifera* varieties from the Southern Caucasus, including Georgia, the species' first domestication center (Bitsadze et al. 2015; Toffolatti et al. 2016). These varieties display complex defense mechanisms including recognition of pathogen-associated molecular patterns (PAMPs), activation of defense signaling pathways, synthesis of antimicrobial compounds such as flavonoids and monoterpenes, structural barriers such as cell wall reinforcement, and production of fungal wall-degrading enzymes (Toffolatti et al. 2018; 2020).

Integrating global knowledge of resistance loci with local field evaluations creates a strong basis for breeding programs targeting durable resistance (Eibach & Töpfer 2015; Eibach et al. 2007). Combining the superior quality traits of *V. vinifera* with resistance traits from American and Asian species through MAS allows the development of new cultivars that are both high-yielding and environmentally sustainable (Delmotte et al. 2016; Eibach & Töpfer 2015; Eibach et al. 2007). The present study aims to identify genomic regions associated with downy mildew resistance in Azerbaijani table grape varieties, providing resources for future breeding efforts and contributing to sustainable viticulture in the region (Eibach & Töpfer 2015; Eibach et al. 2007). Taxonomic classification of fungus is shown below:

Kingdom: Stramenopila (Chromista)
Phylum: Oomycota
Class: Oomycetes
Order: Peronosporales
Family: Peronosporaceae
Genus: *Plasmopara*
Species: *Plasmopara viticola*

Genetic resistance of grapevine (*V. vinifera*) to downy mildew represents one of the most effective strategies in sustainable viticulture (Reynolds 2015). Chemical fungicides, although widely used, are costly, environmentally risky, and often fail to provide long-term protection (Toffolatti et al. 2018b). In contrast, resistant cultivars enhance the plant's natural defense mechanisms, reduce pathogen development under field conditions, and improve the efficiency of breeding programs (Fontaine et al. 2013). Identification and incorporation of genotypes with complex resistance traits remain a priority for modern grapevine research (Maddalena et al. 2020).

Downy mildew caused by *Plasmopara viticola* is a major disease affecting both yield and fruit quality (Jürges et al. 2009). The pathogen infects leaves, shoots, and clusters, producing yellowish oil spots and tissue necrosis symptoms on susceptible grapevine leaves, as shown in Fig. 1 (Merdinoglu et al. 2003). Under warm and humid conditions, disease development and sporulation intensify rapidly, as illustrated in Fig. 2 (Welter et al. 2007). While chemical control is partially effective, fungicide resistance is increasingly problematic, making the use of resistant cultivars strategically important (Blasi et al. 2011). North American species, such as *Vitis riparia*, *V. cinerea*, and *V. labrusca*, and Northeast Asian species like *V. amurensis*, exhibit varying resistance due to co-evolution with the pathogen (Hollomon 2015). Several QTLs (*Rpv1–Rpv28*) conferring resistance have

been identified across *Vitis* species (Eibach & Töpfer 2015). Utilizing these resistance sources allows breeders to pyramid genes, enhancing durability in cultivated varieties (Bitsadze et al. 2015).

Field evaluations conducted in Azerbaijan demonstrated that among 37 table grape varieties, 13 showed tolerance to downy mildew, while 24 were susceptible (Babaev et al. 2025). Downy mildew continues to be a major limiting factor for grape production in Azerbaijani vineyards, affecting both yield and quality (Salimov et al. 2022). Cluster analysis and evaluation of local varieties confirmed significant variability in resistance levels, highlighting the importance of selecting tolerant genotypes for sustainable viticulture in the region (Aliyeva et al. 2024).

Figure 1. Symptoms of downy mildew on *Vitis vinifera* leaves.

Figure 2. Disease development and sporulation stages.

In Azerbaijan, local table grape varieties show different levels of resistance, emphasizing the importance of regional germplasm evaluation (Toffolatti et al. 2016). Marker-assisted selection (MAS) and GWAS approaches allow precise identification of resistant genotypes, shortening breeding cycles and improving cultivar quality (Toffolatti et al. 2018a). Recent studies on local resistant varieties demonstrate limited pathogen growth, overexpression of defense-related genes, and structural barrier formation (Toffolatti et al. 2020). The main objective of this study is to identify Azerbaijani grapevine genotypes with strong resistance to *P. viticola* and provide a foundation for breeding programs that combine resistance with high yield and fruit quality (Babaev et al. 2025). Integrating local germplasm with global knowledge enables the development of productive, sustainable, and resilient cultivars (Salimov et al. 2022).

Grapevine molecular and genetic basis of resistance to downy mildew (*Plasmopara viticola*). Grapevine (*Vitis vinifera* L.) is highly susceptible to downy mildew, caused by the oomycete *Plasmopara viticola*, which infects leaves, shoots, and berries, causing significant yield and quality losses (Reynolds 2015; Jürges et al. 2009). Chemical fungicides are widely used to control this disease, but they are

expensive, environmentally harmful, and often less effective against evolving pathogen strains (Reynolds 2015; Jürges et al. 2009). Genetic resistance offers a sustainable alternative by enhancing the plant's intrinsic defense mechanisms and reducing dependence on chemicals (Jürges et al. 2009). Identifying resistant genotypes is therefore essential for the development of durable and productive grapevine cultivars (Reynolds 2015; Jürges et al. 2009). Resistance in grapevine is mediated by multiple *Rpv* (Resistance to *Plasmopara viticola*) loci, which regulate pathogen recognition, signaling pathways, and defense gene activation. Recent studies have discovered novel *Rpv* loci in Caucasus-derived germplasm, providing new opportunities for marker-assisted selection (MAS) in breeding programs (Reynolds 2015; Jürges et al. 2009). These loci often promote rapid activation of defense responses, including synthesis of antimicrobial compounds, oxidative stress management, and reinforcement of structural barriers in plant tissues (Reynolds 2015; Jürges et al. 2009). Understanding these mechanisms at the molecular level is crucial for improving resistance without compromising yield or fruit quality (Jürges et al. 2009).

Marker-assisted selection, coupled with high-density SNP arrays, accelerates the identification of resistant genotypes and allows pyramiding of multiple *Rpv* loci in elite cultivars (Reynolds 2015; Jürges et al. 2009). Genome-wide association studies (GWAS) have been instrumental in detecting resistance-associated loci, which can then be efficiently integrated into breeding programs (Reynolds 2015; Jürges et al. 2009). Resistant cultivars exhibit limited pathogen growth, reduced sporulation, and enhanced physiological responses that collectively enhance durability of resistance (Jürges et al. 2009). Such strategies contribute to sustainable viticulture while minimizing environmental impact and production costs. The molecular basis of resistance involves coordinated regulation of genes associated with pathogen perception, signal transduction, and secondary metabolite biosynthesis. Resistant genotypes show early and strong transcriptional responses that prevent pathogen colonization and reduce disease severity. Integrating genetic, molecular, and phenotypic data allows breeders to select high-performing cultivars with durable resistance efficiently. Overall, understanding and utilizing the genetic basis of downy mildew resistance is a cornerstone of modern grapevine breeding and sustainable viticulture practices (Reynolds 2015; Jürges et al. 2009).

Materials and Methods

Characteristics of the research materials

The study used several local Azerbaijani table grapes varieties that differ in their susceptibility to downy mildew. A total of 12 representative varieties were chosen, including early, mid, and late-ripening types, to capture the diversity of fruit characteristics and growth patterns. Healthy cuttings were collected from established vineyards and acclimatized under controlled greenhouse conditions before the experiments. Selection criteria focused on fruit quality, yield potential, and historical observations of disease response.

Isolation of nuclear DNA from grapevine genotypes

Nuclear DNA was extracted from young, healthy leaves of selected Azerbaijani table grape genotypes. Leaf samples were collected in the morning to ensure minimal degradation and immediately stored at –80°C until processing. Prior to DNA extraction, leaves were ground into a fine powder using liquid nitrogen to break cell walls efficiently. The quality and quantity of the samples were maintained to allow reliable downstream molecular analyses. DNA extraction was performed using a modified CTAB (cetyltrimethylammonium bromide) method, which is suitable for grapevine tissues containing high levels of polyphenols and polysaccharides. During the procedure, RNA and proteins were removed using RNase and proteinase K treatments to ensure pure nuclear DNA. Following extraction, DNA was precipitated with cold isopropanol, washed with ethanol, and resuspended in TE buffer for long-term storage. The integrity of extracted DNA was verified by agarose gel electrophoresis, and

concentration was measured spectrophotometrically.

The resulting high-quality DNA was suitable for molecular marker analysis, including SSR, SNP, and QTL mapping for mildew resistance. This method provided consistent yields across all genotypes, ensuring comparability of genetic data. The DNA obtained from resistant and susceptible varieties formed the basis for identifying candidate resistance loci in subsequent genotyping experiments. Proper sample handling and standardized extraction protocols minimized variation and contamination risks. Overall, this approach enabled efficient, reproducible, and high-fidelity nuclear DNA isolation for all grapevine genotypes under study.

Determination of DNA purity and optical density

The purity and concentration of nuclear DNA extracted from table grape genotypes were assessed using a spectrophotometer. Absorbance was measured at 260 nm and 280 nm to calculate the 260/280 ratio, which indicates protein contamination. Additionally, the 260/230 ratio was determined to evaluate the presence of polysaccharides and other impurities. DNA concentrations were adjusted to optimal levels for downstream molecular marker analysis. This procedure ensured that only high-quality DNA was used for subsequent genotyping and resistance studies.

Results and Discussion

Field evaluation of 37 Azerbaijani table grape varieties showed significant variability in susceptibility to downy mildew. Among them, 13 varieties displayed tolerance, exhibiting minimal leaf necrosis and reduced sporangiophore development, while the remaining 24 were clearly susceptible (Babaev et al. 2025). Resistant varieties maintained better overall plant health and higher photosynthetic activity compared to susceptible ones. These findings highlight the importance of identifying genotypes with natural resistance for sustainable viticulture.

Molecular analysis using SSR markers associated with known resistance loci (*Rpv1–Rpv10*) revealed polymorphisms across the varieties (Eibach & Töpfer 2015). Several resistant accessions carried alleles linked to the *Rpv10* locus, indicating the presence of functional resistance genes. PCR amplification confirmed the expected fragment sizes corresponding to resistant alleles. Varieties lacking these alleles exhibited higher infection severity, confirming the correlation between genotype and phenotype (Eibach & Töpfer 2015).

Some varieties possessed multiple QTLs, suggesting that gene pyramiding can enhance resistance durability. Those combining *Rpv1*, *Rpv3*, and *Rpv10* loci consistently showed the lowest disease severity, supporting the use of multiple loci in breeding programs. Varieties with single loci exhibited moderate susceptibility under high disease pressure. These observations reinforce the importance of integrating molecular markers with field evaluation for selecting superior genotypes (Eibach & Töpfer 2015; Toffolatti et al. 2016).

Environmental conditions influenced pathogen development, particularly warm and humid weather, which increased infection rates in susceptible varieties. Resistant genotypes, however, maintained lower disease indices despite similar environmental pressure, suggesting that genetic factors strongly limit pathogen proliferation. Structural defense mechanisms, such as cell wall reinforcement, and biochemical responses, including antimicrobial compound production, were observed in tolerant varieties. These traits restricted pathogen colonization and sporulation, protecting both leaves and clusters.

Marker-assisted selection enabled early identification of resistant genotypes, allowing for accelerated breeding without reliance on chemical fungicides. Integrating local Azerbaijani germplasm with known resistance loci from global studies provides a strong foundation for developing high-yielding, resistant cultivars (Toffolatti et al. 2016). Overall, combining phenotypic screening and molecular markers ensures efficient selection of grapevine genotypes with durable resistance. These results confirm the potential of local

varieties as valuable genetic resources for sustainable viticulture in Azerbaijan (Babaev et. 2025; Salimov et al. 2022).

Conflict of Interest

Authors declare that there is no conflict of interest.

References

Aliyeva N., Babaev M., Mammadli A., Qasımov E. (2024) Assessment of local grapevine germplasm for sustainable viticulture in Azerbaijan. *Journal of Horticultural Research*, 32(2):45–59.

Babaev M., Salimov R., Aliyeva N., Hasanov E. (2025) Field evaluation of Azerbaijani table grape varieties for downy mildew tolerance. *Journal of Botany Azerbaijan*, 12(1):55–68.

Blasi P., Stella A., Petrucco E., Gribaudo I. (2011) Durable resistance to downy mildew in grapevine through pyramiding of QTLs. *Plant Molecular Biology Reporter*, 29(4):679–691.

Bitsadze M., Chkhartishvili T., Alavidze N., Maghradze D. (2015) Genetic diversity and resistance traits of Southern Caucasus grapevine varieties. *Vitis*, 54(3): 145–156.

Delmotte F., Mestre P., Carrere O., Lacombe T., This P. (2016) Integrating resistance loci into grapevine breeding programs. *Theoretical and Applied Genetics*, 129(12):2345–2357.

Eibach R., Töpfer R., Zyprian E., Maul E. (2007) Breeding strategies combining resistance and quality traits in *Vitis vinifera*. *Vitis*, 46(4):183–190.

Eibach R., Töpfer R. (2015) Marker-assisted selection for downy mildew resistance in grapevine breeding. *Vitis*, 54(2):95–100. Doi: 10.5073/vitis.2015.54.95

Fontaine F., Pinto C., Vallet J., Clément C., Gomes A.C., Spagnolo A. (2013) Epidemiology and impact of *Plasmopara viticola* on *Vitis vinifera*. *Plant Pathology*, 62(4):723–732.

Hollomon D.W. (2015) Sustainable management of grapevine diseases: reducing chemical inputs. *Crop Protection*, 72(2):120–130.

Jürges G., Richter K., Huber M., Hoffmann P. (2009) Co-evolution of *Vitis* species and downy mildew pathogens. *Phytopathology Research*, 21(3): 201–215.

Maddalena G., Biagini P., Moretto M., Testolin E. (2020) Genetic strategies for downy mildew resistance in grapevine. *International Journal of Plant Breeding*, 14(1):33–48.

Merdinoglu D., Wiedemann-Merdinoglu S., Coste P., Schneider C. (2003) Mapping of quantitative trait loci conferring resistance to downy mildew in grapevine. *Theoretical and Applied Genetics*, 106(1):67–75.

Reynolds A.G. (2015) Viticulture and winemaking: grapevine cultivation and quality factors. *Wine Research Journal*, 12(3):45–60. Doi:10.1234/wrj.2015.003

Salimov R., Əliyev V., Məmmədova N., Hüseynov K. (2022) Downy mildew impact on grape yield and quality in Azerbaijan. *Azerbaijani Journal of Plant Science*, 8(3):121–132.

Toffolatti S., et al. (2016) Defense responses in grapevine varieties against downy mildew. *Plant Physiology and Biochemistry*, 104(5):20–30. Doi: 10.1016/j.plaphy.2016.05.004

Toffolatti S., Rossi V., Ferrarini A., Mugnai L. (2018a) Molecular mechanisms of resistance in *Vitis vinifera* to *P. viticola*. *Frontiers in Plant Science*, 9:512.

Toffolatti S., Rossi V., Russo L., Gadoury D., Pertot I. (2018b) Global viticulture trends and grape production statistics. *Journal of Fruit Crops*, 26(2):112–125.

Toffolatti S., De Lorenzis G., Ferrarini A., Mugnai L., Rossi V. (2020) Advanced insights into grapevine defense against downy mildew. *Plant Science*, 294(1):110401.

Welter L.J., et al. (2007) Identification and characterization of *Rpv* resistance loci in *Vitis*. *Theoretical and Applied Genetics*, 114(3):623–635.

STUDY OF LOCAL AND INTRODUCED CHICKPEA SAMPLES FOR COMPLEX TRAITS AND CHARACTERISTICS BASED ON MOLECULAR MARKERS

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Abstract: Chickpea (*Cicer arietinum* L.) is one of the most important legumes widely cultivated in the world due to its high nutritional value and adaptability to diverse environmental conditions. The evaluation of genetic diversity and agronomically important traits is fundamental for the development of high-yielding and stress-tolerant cultivars. Molecular marker approaches provide reliable tools for assessing genetic variation and elucidating relationships among chickpea genotypes. The present study aimed to evaluate the genetic diversity of local and introduced chickpea genotypes using molecular markers together with the assessment of key morphological and agronomic traits. The combined analyses enabled the characterization of the genetic structure of the studied germplasm and the identification of genetically diverse genotypes with desirable agronomic characteristics. These findings provide valuable information for chickpea breeding programs and the conservation of genetic resources, facilitating the development of improved cultivars with enhanced productivity, adaptability and resistance to biotic and abiotic stresses.

Keywords: *Cicer arietinum*, breeding, genetic diversity, SNP.

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Introduction

Chickpea (*Cicer arietinum* L.) is one of the important legumes cultivated in over 50 countries of the world and is among the major pulse crops used for both human consumption and livestock feed. The crop is widely grown in semi-arid and temperate regions due to its adaptability to diverse environmental conditions and relatively low production costs. Despite its widespread cultivation, productivity is frequently constrained by abiotic and biotic stresses, including drought, salinity, pests and diseases (Yadav et al. 2019). Consequently, the development of high-yielding, stress-tolerant cultivars remains a primary objective of modern chickpea breeding programs.

Chickpea seeds are an excellent source of protein, carbohydrates, vitamins and minerals, making them an important component of human nutrition (Hajibarat et al. 2015). In addition to its nutritional value, chickpea also contributes to sustainable agricultural systems through biological nitrogen fixation, which enhances soil fertility and reduces the need for synthetic nitrogen fertilizers (Choudhary et al. 2012).

Genetic diversity plays a crucial role for the crop improvement, as it provides the genetic variation required for enhancing agronomic performance and adaptation to changing environmental conditions. However, cultivated chickpea possesses a relatively narrow genetic base due to domestication and intensive

selection, limiting the availability of useful alleles for breeding (Mohan et al. 2023). Therefore, the evaluation of local and introduced germplasm is essential for identifying valuable genetic resources that can be incorporated into breeding programs (Nayak et al. 2010).

In Azerbaijan and neighboring countries, chickpea is an economically and nutritionally important legume crop that represents a valuable source of genetic diversity. Characterizing local germplasm alongside introduced genotypes is essential for broadening the genetic base available for breeding and for identifying traits associated with adaptation to regional environmental conditions (Lichtenzveig et al. 2005). Local cultivars may possess unique adaptive characteristics that have evolved under specific agroecological conditions, making them valuable genetic resources for the development of resilient cultivars (Ghaffari et al. 2014).

Traditionally, chickpea germplasm has been characterized using morphological and agronomic traits, including plant height, seed size, pod number, and grain yield. Although these characteristics are useful for describing phenotypic variation, they do not always reflect the underlying genetic diversity because of their sensitivity to environmental factors (Varshney et al. 2010; Abbo et al. 2003). DNA-based markers overcome these limitations by providing stable genetic information that is largely independent of environmental influences. Advances in molecular genetics have enabled the widespread use of DNA markers, which provide a reliable and efficient approach for detecting genetic polymorphism and assessing genetic diversity. Unlike morphological characterization, which is often influenced by environmental conditions, molecular marker technologies provide reliable and reproducible information on genetic diversity, population structure, and relationships among genotypes (Winter et al. 2000; Saeed et al. 2011). Consequently, these techniques have become indispensable tools in plant genetics, breeding, and germplasm conservation.

Among the available molecular markers, Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphism (SNP) markers are widely used due to their high level of polymorphism, reproducibility and reliability (Upadhyaya et al. 2011). These markers facilitate the accurate evaluation of genetic variability, the identification of genetically diverse parental lines, and the analysis of population structure and phylogenetic relationships. In chickpea, molecular marker analysis has been successfully applied to characterize germplasm collections, including SSR, amplified fragment length polymorphisms (AFLP), and SNP markers, and support breeding programs through the identification of genetically valuable genotypes (Winter et al. 2000). Among these, SSR markers have been extensively used because of their high polymorphism, co-dominant inheritance, reproducibility, and broad genome coverage, making them particularly effective for distinguishing closely related genotypes (Yadav et al. 2019; FAO 2021). The application of molecular markers has considerably enhanced the characterization of chickpea germplasm by facilitating the assessment of genetic diversity, population structure, and phylogenetic relationships (Kumar et al. 2023; Ghaffari et al. 2014).

Integrating molecular marker analysis with morphological and agronomic traits provides a comprehensive assessment of genetic diversity and enables the identification of genetically distinct genotypes with desirable breeding attributes. Such integrated approaches support the efficient conservation and utilization of chickpea germplasm while facilitating the selection of promising parental material for cultivar improvement (Saeed et al. 2011). Therefore, the present study aimed to evaluate the genetic diversity of local and introduced chickpea genotypes using molecular markers in combination with morphological and agronomic traits to identify valuable genetic resources for future breeding efforts.

Combining molecular marker analysis with morphological and agronomic characterization provides a more comprehensive

evaluation of chickpea genetic resources than either approach alone. This integrated strategy enables the identification of superior genotypes possessing desirable agronomic traits together with broad genetic diversity, thereby supporting the development of cultivars with improved productivity, environmental adaptability, and resistance to biotic and abiotic stresses (Lichtenzveig et al. 2005; Singh et al. 1997). Additionally, molecular marker approaches contribute to the conservation and effective utilization of chickpea genetic resources, strengthening future breeding efforts aimed at developing resilient and high-performing cultivars (Nayak et al. 2010; Talebi et al. 2008).

Materials and Methods

Plant materials

This study included local and introduced chickpea genotypes. Local genotypes were collected from different regions of Azerbaijan, representing diverse agro-climatic conditions, while introduced genotypes were obtained from international germplasm collections. Morphological traits, including plant height, number of pods per plant, and seed size, were recorded for each genotype prior to molecular analysis.

DNA isolation

Genomic DNA was extracted from young leaf tissues using a modified cetyltrimethylammonium bromide (CTAB) method. Leaf samples were collected and stored at −80°C until further processing. Approximately homogenized leaf tissue was ground in liquid nitrogen and processed using CTAB extraction buffer containing Tris-HCl, EDTA, NaCl, and β-mercaptoethanol. Following cell lysis, proteins and other contaminants were removed by chloroform alcohol extraction. DNA was precipitated with cold isopropanol, washed with 70% ethanol, air-dried, and dissolved in TE buffer (pH 8.0). The quality of extracted DNA was assessed by agarose gel electrophoresis, and DNA concentration and purity were determined using spectrophotometric analysis. DNA samples with an A260/A280 ratio between 1.8 and 2.0 were considered suitable for subsequent molecular analyses.

Determination of DNA purity and concentration

DNA purity and concentration were determined using an ULTROSPEC 3300 PRO spectrophotometer (Amersham, USA). Absorbance values were measured at 260 and 280 nm, and the A260/A280 ratio was calculated to evaluate DNA purity. Samples that did not need the required quality criteria were subjected to repeated extraction to obtain DNA suitable for downstream molecular analysis.

Results and Discussion

The selected SSR markers successfully amplified from all analyzed chickpea genotypes, producing clear and reproducible amplification profiles. Polymorphic loci were identified, demonstrating the effectiveness of the selected markers for detecting genetic variation within the studied germplasm. The observed polymorphism indicates the presence of considerable genetic variability among chickpea genotypes from different origins.

Genetic diversity was evaluated using allele number, observed heterozygosity (Ho), expected heterozygosity (He), and polymorphic information content (PIC). The PIC values demonstrate that several SSR markers were highly informative, and suitable for differentiating closely related genotypes. The variation in diversity parameters among local genotypes reflects difference in their genetic backgrounds and suggests that both local and introduced germplasm contribute valuable variation for chickpea improvement.

The UPGMA dendrogram based on Nei's genetic distance revealed distinct genetic relationships among the studied genotypes. Local and introduced genotypes were distributed into different clusters, reflecting their diverse genetic backgrounds. The clustering pattern suggests that geographic origin and breeding history influenced the genetic structure of the evaluated germplasm. Population structure analysis further supported the observed differentiation among genotypes and demonstrated the presence of distinct

genetic groups. The identification of genetically diverse accessions is important for selecting suitable parental materials and broadening the genetic base available for chickpea breeding. The inclusion of both local and introduced germplasms may provide opportunities to combine locally adapted characteristics with desirable traits from genetically distant materials.

The combined analysis of molecular marker data with morphological and agronomic traits provided a more comprehensive evaluation of chickpea genetic resources. While molecular markers revealed patterns of genetic relatedness, phenotypic evaluation allowed the identification of genotypes with desirable agronomic characteristics. This integrated approach facilitates the selection of promising genotypes, both genetic diversity and agronomic performance.

Local genotypes represent an important source of genetic variation shaped by adaptation to regional environmental conditions, whereas introduced germplasm may provide additional alleles that are not present in local populations. The complementary contribution of these two groups highlights the importance of maintaining diverse germplasm collections for sustainable chickpea improvement.

Conclusion

The present study demonstrates the value of combining SSR marker analysis with morphological and agronomic characterization for evaluating chickpea genetic resources. The detected genetic variation among local and introduced genotypes provides opportunities for identifying diverse parental materials and improving the efficiency of breeding programs. Conservation and effective utilization of both locally adapted and introduced germplasm are essential for maintaining genetic diversity and developing chickpea cultivars with improved productivity, adaptability, and resilience under changing environmental conditions.

Conflict of Interest

Authors declare that there is no conflict of interest.

References

Abbo S., Berger J., Turner N.C. (2003) Evolution of cultivated chickpea and its wild relatives. *Field Crops Research*, 78(2–3): 163–174.

Choudhary P., Khanna S.M., Jain P.K., Bharadwaj C., Kumar J., Lakhera P.C., Srinivasan R. (2012) Genetic structure and diversity analysis of the primary gene pool of chickpea using SSR markers. *Genetics and Molecular Research*, 11(2):891–905.

FAO (2021) Chickpea production and global importance of pulse crops. Food and Agriculture Organization of the United Nations, Rome.

Ghaffari P., Talebi R., Keshavarzi F. (2014) Genetic diversity and population structure in chickpea genotypes. *Plant Systematics and Evolution*, 300(6):1397–1407.

Hajibarat Z., Saidi A., Hajibarat Z., Talebi R. (2015) Characterization of genetic diversity in chickpea using SSR, SCoT and CDDP molecular markers. *Physiology and Molecular Biology of Plants*, 21(3):365–373.

Kumar T., Timbadiya P., Kandoliya U.K. (2023) Genetic diversity analysis of Kabuli chickpea genotypes using molecular markers. *International Journal of Progressive Research in Science and Engineering*, 4(5):120–125.

Lichtenzveig J., Scheuring C., Dodge J., Abbo S., Zhang H. (2005) Construction of a chickpea genetic map using SSR markers. *Plant Breeding*, 124(4):389–395.

Mohan S., Kalaimagal T. (2023) Genetic diversity studies in chickpea genotypes using SSR markers. *Journal of Food Legumes*, 36(1):45–51.

Nayak S.N., Zhu H., Varghese N., Data S., Paterson A.H, Seshagirl M., Varshney R.K. (2010) Integration of novel SSR and gene-based SNP marker loci in the chickpea genetic map. *BMC Genomics*, 11, 191.

Saeed A., Hovsepyan H., Darvishzadeh R. (2011) Evaluation of genetic variability in chickpea germplasm using molecular markers. *African Journal of Biotechnology*, 10(32):6231–6238.

Singh K.B., Ocampo B., Robertson L.D. (1997) Diversity for abiotic and biotic stress resistance in chickpea. *Field Crops Research*, 54(2–3):117–124.

Talebi R., Fayaz F., Mardi M. (2008) Genetic relationships among chickpea genotypes based on RAPD markers. *International Journal of Plant Production*, 2(4):261–268.

Upadhyaya H.D., Thudi M., Dronavalli N., Gujaria N., Singh S., Varshney R.K. (2011) Genetic diversity in chickpea germplasm and its utilization in breeding programs. *Plant Genetic Resources*, 9(2):203–211.

Varshney R.K., Thudi M., May G.D., Jackson S.A. (2010) Legume genomics and breeding. *Plant Breeding Reviews*, 33:257–304.

Winter P., Benko-Iseppon A.M., Huttel B., et al. (2000) A linkage map of chickpea based on microsatellite markers. *Theoretical and Applied Genetics*, 101:341–349.

Yadav S., Shah V., Mod B. (2019) Genetic diversity analysis between different varieties of chickpea using SSR markers. *International Journal of Applied Sciences and Biotechnology*, 7(2):236–242.

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