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PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF WHEAT TO VIRUSES AND CLIMATE CHANGE

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Summary. We conducted monitoring for wheat viruses in Ukraine. We identified the presence of *Wheat streak mosaic virus*, *Barley yellow dwarf virus* and *Wheat dwarf virus*, all of which have a significant epidemic potential, leading to reduced crop yields and compromised quality. Additionally, we observed "cold peaks" due to a temperature drop of more than 18ºC, leading to stress symptoms such as leaf reddening and the development of chlorotic and yellowish-brown stripes. It was studied the wheat physiological-biochemical responses to viruses of several varieties according to parameters of ROS homeostasis, chlorophyll and saccharide levels and the activity of protective proteins.

Keywords: wheat, viruses, plant biochemical protective responses

Introduction

Wheat (*Triticum aestivum* L.), as the paramount staple food and commodity crop in Ukraine, holds immense economic significance while simultaneously serving as a critical contributor to global food security. Regrettably, the russian invasion has cast a long shadow over Ukraine's wheat research and development endeavors, precipitating far-reaching consequences that ripple across global food systems. These disruptions compound the preexisting crises stemming from the COVID-19 pandemic and the surge in food prices, imperiling millions' livelihoods, particularly in nations heavily reliant on wheat. This has resulted in an escalation of wheat prices, which has increased the prevalence of undernutrition and undermined progress towards reaching the Sustainable Development Goals (SDGs). The present precarious situation emphasizes the urgent need to address wheat production's challenges, including the multifaceted effects of biotic and abiotic stresses, climatic changes, and edaphic factors. In this context, the escalation of diseases such as viruses poses a particularly concerning threat to sustainable wheat production.

The *Wheat streak mosaic virus* (WSMV), *Barley yellow dwarf virus* (BYDV), *Wheat dwarf virus* (WDV) are among the most serious diseases of wheat. The issue of climate change is also very relevant, because in recent years, abnormal climatic phenomena have been observed, which create several significant problems for grain production. Therefore, the problem of assessing the impact of expected climate changes on agroclimatic growing conditions, productivity and gross harvest of agricultural crops is extremely relevant (Mishchenko, 2018). Furthermore, global climate changes also increase the impact of diseases on the defeat of plants to viruses.

An important role in solving these issues can be obtained by the study of physiological and biochemical plant responses to viruses and climate change. Biotic and abiotic stresses induce physiologic, transcriptomic and metabolomic changes in plants (Backes et al., 2021). In the process of evolution, plants have developed various protective mechanisms, which occur at different levels

– from the genome (differentiated expression of genes, including PR genes) through the proteome (production of protective proteins and enzymes) to the metabolome (changes in the activity of several metabolic pathways, including carbohydrate metabolism, phenolic metabolism, etc.) (Kaur et al., 2022). An important system that lies at the heart of the cellular and molecular mechanisms of plant adaptation to unfavorable environmental factors and determines the direction of the general adaptive response is the system of ROS homeostasis (Dumanovic et al., 2021). It is known that chitinases and β-1,3-glucanases are markers of plant protective responses to various pathogens.

The goal of our research was the identify the viruses on wheat crops in Ukraine and study the influence of viruses (*Wheat streak mosaic virus* (WSMV), *Barley yellow dwarf virus* (BYDV), *Wheat dwarf virus* (WDV) and cold stress on physiological and biochemical plant responses (the parameters of ROS homeostasis, chlorophyll and saccharide levels and the activity of protective proteins) in several varieties of wheat.

Material and methods

Phytopathological monitoring of viruses was carried out on winter wheat crops in different regions of Ukraine in 2023 and 2024 years.

The following varieties of wheat were used in the research: 'Patras', 'Pamir', 'Jersey', 'Shestopalivka', 'Staleva', 'Lisova pisnia', 'Bohdana', 'Kolonia' and 'Dagmar'.

Protein content was determined by the Kjeldahl method (Kjeldahl, 1983). Total carbohydrate content was measured with the anthrone method (Hedge and Hofreiter, 1969). Chlorophyll content was determined using a spectrophotometric method (Dashek and Miglani, 2017). Flavonoid content of the extracts was quantified using the aluminium chloride assay method (Chepel et al., 2020). Hydrogen peroxide (H_2O_2) concentration was determined in the supernatant (Sagisaka, 1976). Lipid peroxidation level was determined fluorometrically using the thiobarbituric acid-reactive substance malondialdehyde (MDA), which forms a 1:2 adduct with thiobarbituric acid (TBA) (Hodges et al., 1999). Reduced glutathione (GSH) content was measured using Ellman reagent (Sigma-Aldrich, Milwaukee, WI, USA) (Hu, 1994). Catalase was measured spectrophotometrically according to Aebi (1984). Peroxidase activity was measured using 4-methylcatechol as substrate (Onsa et al., 2004). Glutathione peroxidase activity was measured by Rotruck et al., 1973. β-1,3-glucanase activity was assayed spectrophotometrically with laminarin as substrate (Sigma L-9634) using the dinitrosalicylic acid (DNS) method (Zur et al., 2013). Chitinase activity was assayed spectrophotometrically with colloidal chitin as substrate (Saikia et al., 2005).

Data processing was performed using Microsoft Office Excel v.14.7.3 (Microsoft, Redmond, WA, USA) or Prism Graph 8 (GraphPad Software, San Diego, CA, USA). Differences between mean values of traits were evaluated by the Student's t-test at probability level $p \leq 0.05$.

Results and discussions

At the visual studies of winter wheat plants of 'Patras', 'Pamir', 'Jersey', 'Shestopalivka', 'Staleva', 'Lisova pisnia', 'Bohdana', 'Kolonia' and 'Dagmar' varieties, significant differences between healthy and damaged plants were found. Using DAS-ELISA and PCR methods it was established that plants of varieties 'Patras', 'Pamir', 'Shestopalivka' were infected by WSMV; plants of variety 'Staleva' – by WSMV, BYDV and WDV and plants of variety 'Jersey' – by BYDV, plants of varieties 'Kolonia', 'Dagmar' – by WDV.

Infection of WSMV, WDV and BYDV causes a significant decrease the content of protein, chlorophylls a and b, carotenoids in the leaves of affected wheat plants in comparison with healthy plants.

Redox homeostasis plays an essential role in plant immunity against viruses. Infection with WSMV increased H_2O_2 content and lipid peroxidation in wheat plants, as evidenced by higher MDA levels. We detected activation of catalase and ascorbate peroxidase by 39% and 51% relative to control in variety 'Jersey' and by 54% and 41% in variety 'Shestopalivka', respectively.

The concentration of WSMV was greater in leaves of the variety ‘Pamir’ than in variety ‘Patras’ suggesting a weaker immune response. As total peroxidase activity and reduced glutathione content in variety ‘Patras’ were higher than in variety ‘Pamir’, both mechanisms are likely to play some role in reducing oxidative damages in the latter genotype. Increase of the ascorbate peroxidase activity in variety ‘Patras’ and decrease of this activity in variety ‘Pamir’ in response to WSMV suggest that enzyme is a key player in reducing oxidative damages. The content of reduced glutathione in leaves of WSMV-infected plants was significantly higher relative to healthy control in both varieties.

An increase of saccharides content in leaves of WSMV-infected plant of studied varieties indicates that all varieties rely on this component for their immunity. This effect can be a consequence of inhibiting phloem loading or slow phloem flow aimed at reducing the long-distance movement of viruses through the phloem. Saccharides can also strengthen the immune response by inducing the accumulation of anthocyanin, activating PR genes, and/or functioning as antioxidants

Analysis of the pathogenesis-related proteins β-1,3-glucanase and chitinase showed significant differences between the varieties. Activity of β-1,3-glucanase increased in ‘Patras’, but reduced in ‘Pamir’ in response to viral infection; activity of chitinase was also higher in ‘Patras’ than in ‘Pamir’. The increase of β-1,3-glucanase activity in variety ‘Patras’ resembles a typical immune response to viral infection, whereas a significant decrease of this activity in variety ‘Pamir’ suggests the immune response involves reduced plasmodesmata permeability.

Infection with WDV and WSMV led to an increase in H_2O_2 content, with a 2-fold increase in the variety ‘Kolonia’ and a 1.3-fold increase in the variety ‘Dagmar’. These viruses also intensified peroxidation of lipids, resulting in a 1.7-fold increase in ‘Kolonia’ and a 1.2-fold increase in ‘Dagmar’ and activated superoxide dismutase, with a 1.6-fold increase in ‘Kolonia’ and a 1.2-fold increase in ‘Dagmar’. As a result of these infections, the activity of some antioxidant enzymes was reduced, including catalase (1.5-fold in ‘Kolonia’, 2.7-fold in ‘Dagmar’) and ascorbate peroxidase (1.3-fold in ‘Dagmar’). Interestingly, WDV infection increased peroxidase activity by 1.3-fold in ‘Kolonia’ and slightly decreased it in ‘Dagmar’, coupled with a 1.7-fold increase in glutathione peroxidase activity.

These metabolic changes indicate that wheat plants of the studied varieties have different mechanisms of ROS homeostasis control in response to viral infection. Metabolic changes in the plant cell can lead to an imbalance in antioxidant metabolism and significant ROS accumulation and damage to cellular macromolecules and membrane lipids, resulting in protein oxidation, lipid peroxidation and other damage that contributes to the development of symptoms of viral infection. The development of these processes can differ in different genotypes, but in general ROS accumulation during infection plays a dual role by causing virus restriction and also performing a signalling function, inducing accumulation of antioxidants and other protective compounds (Hernandez et al., 2015; Kuzniak and Kopczewski 2020). Development of a protective reaction largely depends on the rate of plant response reaction. In more resistant genotypes, early ROS accumulation at virus localization sites can lead to premature elimination of the virus without the development of oxidative stress through active formation of antioxidants. A slower plant response allows a certain degree of replication and movement of the virus, which leads to oxidative stress and programmed death of affected plant cells (hypersensitivity reaction) with slow activation of antioxidants. In more susceptible varieties, gradual ROS accumulation without antioxidant activation and other defense mechanisms (low levels of protective proteins etc.) leads to massive oxidative stress, which causes damage to plant cells and significant plant injury, a sharp imbalance in the antioxidant system and virus development, resulting in significant yield reductions.

We were not detected viral infection in the varieties ‘Lisova pisnia’ and ‘Bohdana’. The decrease in the content of chlorophylls and carotenoids, as well as the activation of the investigated antioxidant enzymes in these varieties, indicates the response of plants to cold stress. An increase in

the sugar content due to the accumulation of sucrose under the action of cold stress was also revealed.

Conclusions

Thus, the obtained results indicate the activation of physiological and biochemical protective reactions of wheat plants under the influence of viral infection and cold stress. A shift in pro-/antioxidant balance was observed in wheat plants infected with both viral diseases and cold stress, resulting in accumulation of H_2O_2 and lipid peroxidation products. This may act as a signal to activate additional plant defence mechanisms, including induction of antioxidants and PR-proteins, ultimately contributing to plant resilience and wheat yield preservation. However, the specific biochemical response to viruses and abiotic stress will depends on the actual stress factor, and predicting the resilience of a specific genotype to a particular virus or abiotic stress factor will require determination of multiple biochemical characteristics, including parameters of ROS homeostasis, chlorophylls, carotenoids and saccharide content, and protective protein activity.

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