

Pathogen-induced synthesis of calloses and phenolic compounds in winter wheat (*Triticum aestivum* L.) cultivar Renan

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Summary. The amount of pathogen-induced callose and phenolic compounds in winter wheat plants under inoculation with the pathogenic fungus *Pseudocercospora herpotrichoides* was determined. Significant differences in the constitutional and induced accumulation of secondary metabolites in Renan seedlings were shown, which determines their systemic resistance to repeated stress.

Keywords: callose, phenolic compounds, winter wheat, fungus.

Introduction

One of the most important ways to increase the yield of crops is to obtain forms resistant to biotic stress [1]. To obtain a high yield of grain crops, it is necessary to conduct continuous selection based on a sufficient number and diversity of genes [2, 3, 4]. To protect soft wheat varieties from pathogens, introgressive resistance genes localized in foreign translocations are widely used [4].

The genotype of wheat variety Renan contains the translocation resistance gene *Pch1*, which is widely used in breeding to obtain resistant productive varieties of this crop. The mechanisms of realization of the resistance of this gene in the form of accumulation of certain protective substances are not known for certain [5]. The formation of protective structures in plants involved in the successful realization of productive ontogeny has not been studied enough, and the results of these studies are presented in few publications [6]. The presence of the translocated *Pch1* gene in plants significantly inhibited the germination of fungal hyphae [7] compared to lines that did not have this sequence. The increase in the time of infection development observed in varieties with this gene [5] slowed down the speed and efficiency of the development of the *P. herpotrichoides* pathogen [9]. Breeding lines with the presence of the *Pch1* gene have increased resistance to the pathogenic fungus *P. herpotrichoides* [10] and other adverse environmental factors [11], which determines the high yield of grain crops [10]. At the same time, the manifestation of the *Pch1* gene in specific defense responses and the accumulation of certain stress metabolites in plants remains poorly understood [11].

The resistance of plants to biotic and abiotic stress-factors is based on the formation of a complex chain of constitutional and induced protective substances that form mechanical [12] and biochemical [13] immune barriers. The cell wall is the first barrier of plants [11]. Numerous changes occur in cell walls in response to microbial invasion [14]. One of the early reactions of plants to damage of their cell walls by hydrolytic enzymes of pathogens is their strengthening, which occurs due to the accumulation of β -1,3-glucan – callose. It is the most widespread component of cell defence [15]. Callose is deposited in the form of spiraled granules between the outer side of the plasmalemma and the inner layer of the cell wall at the sites of pathogen invasion. In addition to being a mechanical barrier to the germination of the fungal hyphae, callose granules are also a place of accumulation of antimicrobial secondary metabolites, including phenols. Such supramolecular complexes synergistically [16] prevent the pathogen from entering the cell. Phenolic compounds are often synthesized and accumulated in the subepidermal layers of plant

tissues that have been damaged by various stresses, including pathogen invasion [17]. Phenols, entering into a chemical bond with callose faces, contribute to the formation of a stable biochemical and mechanical barrier [18]. As a result of the interaction of this structural and biochemical conglomerate with other components of the cell wall, protective cluster structures are formed, which lead to increased resistance of plant tissues to the action of enzymatic complexes of pathogens during re-invasion [19]. In addition, plant synthesized phenolic compounds are a natural alternative to chemical means of controlling crop pathogenesis [20], which can undoubtedly be used in plant biotechnology to initiate immunity enhancement by pre-priming [21] and hardening [22]. The pathogenesis is caused by the prolonged accumulation of phytoalexins - hydroxycoumarin [23] and others. The latter act as both prolonged-acting fungitoxins in areas of local pathogenesis [24] and substances of induced systemic resistance. Barrier protective clusters are formed on the periphery of necrotic tissues in the reaction of Hypersensitive response (cell death) [25], and at the same time they are an element of the systemic resistance of plants to repeated pathogen damage [26] or other environmental stressors [27]. Recognition of potential pathogenesis signals and their sequential transduction into the cell interior [28] triggers genetically determined immune responses. Plant cells, among other things, synthesis and accumulate phenolic compounds [29], which results in pathogen-associated molecular patterns (PAMPs) that lead to PAMP-mediated immunity [28]. As a result, the development and spread of infection is often limited long before the pathogen can penetrate the inner layers of plant tissues [30].

Phenolic compounds and callose are part of papillae, hemispherical capsule-like formations between the plasmalemma and the cell wall, which are a component of the PTI (pattern-triggered immunity) response. To prevent repeated pathogenesis, plants thicken the cell wall in places of previous contact with the microorganism, forming papillae as an element of systemic resistance [15]. Thus, papilli are supramolecular complexes containing callose, cellulose, lignin, suberin, phenolic polyamines, ROS, arabinogalactan proteins, hydroxyproline, rich glucoproteins, pectin, xyloglucans, as well as inorganic chemical elements such as calcium, silicon, zinc, etc. Due to the cooperative behaviour [32] of the complexes of the protective barrier system - papillae, both the mechanical properties of the cell wall are enhanced and the rapid and effective implementation of the local biochemical defence response of plant cells and tissues under the influence of extracellular enzymatic complexes of pathogens [13], mechanical stress [8], etc. is ensured.

The study of the dynamics of the callose accumulation [8] and phenolic compounds [20] in plants of the *Poaceae* family as essential molecular defences is very relevant. Also, the issues of their quantitative accumulation [33] and the ratio of the amount of certain metabolites of protective action [11] in superorganised structures that appear under stress and provide increased plant resistance to repeated exposure to adverse environmental factors, which is the key to high yields, remain poorly understood. For the experiments, we chose winter wheat plants of the Renan variety with the presence of the *Pch1* gene [33], which is characterised by increased resistance to *P. herpotrichoides* and high yield [27, 32].

The experimental work involved the study of the dynamics of changes in the amount of accumulation of callose and phenolic compounds in winter wheat seedlings of the Renan variety under the pathogenesis of the hemibiotrophic fungus *Pseudocercospora herpotrichoides* (Fron) Deighton and constitutionally. Analysis of the results.

Materials and methods

Seedlings of winter wheat, a relatively resistant to the pathogen *P. herpotrichoides* variety Renan [34], were used in the experiments and grown on sandy culture. The wheat seeds were planted in pots of 100 pieces. Seven-day-old seedlings were infected with a suspension of conidia in the concentration range of 1×10^2 - 1×10^5 CFU (colony forming units)/ml [35] of the highly virulent strain 543 7/1 of from the collection of the Institute of Plant Protection of the National Academy of

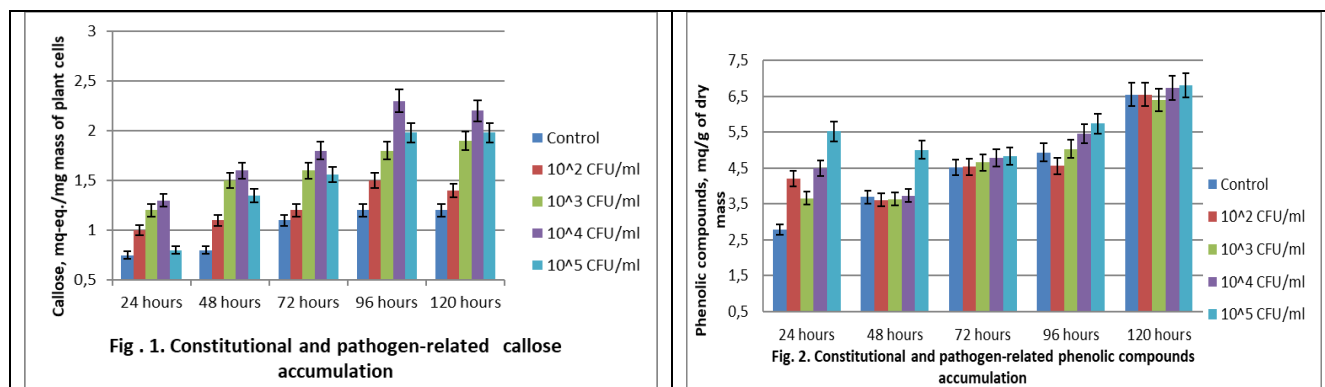
Sciences of Ukraine. Control variants were treated with distilled water. Plant material was sampled every 24 hours for 5 days.

The isolation and quantification of callose was performed according to the modified method of Kauss et al. [36]. Measurements of callose luminescence were performed using the SDL-1 device (LOMO, Russia), excitation wavelength (λ_{ex}) – 397 nm, measurement wavelength (λ_{em}) – 490 nm.

The total content of phenolic compounds was determined using the Folin-Checalt reagent. Ferulic acid, as the dominant phenolic substance in plants of the cereal family [37], was used to construct a calibration curve for calculating the content of total phenolics in ethanol extracts. The material was dried at 60°C in a ventilated oven. 100 mg of the dry material was ground with glass powder and extracted in 1 ml of 95% ethanol for 24 h. The amount of phenolics was determined by the optical density, at $\lambda = 765$ nm, on a spectrophotometer.

Result and discussions

At the first stage of the experimental work, the amount of constitutional callose accumulation in 8 – 12 days-old seedlings of the selected varieties was determined. The results of the experiments showed that after 24 hours of study, the amount of constitutional callose accumulation in Renan seedlings was about 0.7 $\mu\text{g-eq/mg}$ of fresh weight of plant cells (Fig. 1). The amount of its deposition gradually increased over 120 hours. From 24 to 48 and 72 to 96 hours of the experiment in the control variants of Renan seedlings, the average increase in callose was 0.1 $\mu\text{g-eq/mg}$ of fresh weight plant cells(fwpc) per day. Its most intensive increase was observed from 48 to 72 hours of research and averaged 0.3 $\mu\text{g-eq/mg}$ fwpc . From 96 to 120 hours of the experiment, its amount remained at the level of the previous day. After inoculation of Renan seedlings with highly virulent pathogen strain 543 7/1 of *P. herpotrichoides*, a gradual increase in induced accumulation of callose was observed, which occurred in most experimental plants in proportion to the increase in the concentration of fungal colonies, except for plants treated with inoculum at a concentration of 10^5 CFU/ml. The increase in callose at 24 hours of the experiment ranged from 0.8 to 1.3 $\mu\text{g-eq/mg}$ of fresh weight in a sequence according to the inoculum concentration: $10^5 < 10^2 < 10^3 < 10^4$ CFU/ml. The amount of pathogen-induced accumulation of callose increased over time from 0.7 to 2.4 $\mu\text{g-eq/mg}$ fwpc and on day 4 of the experiment it was from 1.5 to 2.4 $\mu\text{g-eq/mg}$ fwpc in accordance with the inoculum concentration: $10^2 < 10^3 < 10^5 < 10^4$ CFU/ml (Fig. 1). On day 5, its amount remained at the same level. The increase in the amount of accumulated callose in Renan seedlings was almost proportional to the increase in the concentration of fungal inoculum (Fig. 1). This dependence was maintained throughout the experiment, and the ability of the pathogen to induce an increase in the amount of its accumulation within 4 – 5 days of concentration was as follows: $10^4 > 10^5 > 10^3 > 10^2$. Thus, the amount of callose accumulated by Renan seedlings increased with increasing pathogen concentration up to 10^4 CFU/ml. Further increase in the fungus concentration did not lead to an increase in the amount of its accumulation in plants. That is, for plants of this variety, at this level of biotic stress, this reaction reached its physiological maximum [38] and was not effective for plant protection and adaptation to this type of stress with further increase in the pathogen concentration.



The next stage of the experiments was devoted to determining the amount of accumulated phenolic compounds under pathogen inoculation in the concentration range of $10^2 - 10^5$ CFU/ml. The results of the experiments on the pathogen-induced amount of accumulated phenolic compounds in Renan variety showed that on the first day of the experiment, their average amount in plants increased proportionally to the increase in the pathogen concentration in the range of : $10^2 - 10^5$ CFU/ml and averaged from 3.6 to 5.6 mg/g of dry biomass, respectively (Fig. 2). At 48 hours of the experiment, there was a slight decrease in their number in all experimental plants. The amount of accumulated compounds in seedlings treated with fungal inoculum at a concentration of $10^2 - 10^4$ CFU/ml averaged 3.6 – 3.8 mg/g dry biomass, which almost coincided with the values in the control. In wheat plants of Renan variety treated with fungal inoculum at a concentration of 10^5 CFU/ml, the amount of phenols decreased on average from 5.6 to 5 mg/g dry biomass at 48 h of the experiment and continued to decrease on average to 4, 8 mg/g dry biomass by 72 h of the study, in contrast to Renan seedlings treated with a suspension of fungal conidia at a concentration of $10^2 - 10^4$ CFU/ml, the amount of which again increased to 4.4 – 4.8 mg/g dry biomass by 72 h of the experiment (Fig. 2).

The obtained results suggest that at a sufficient level of pathogenesis in Renan seedlings after 1 day of the experiment, where the most intense surge of phenol accumulation was observed, their transformation into other molecular conglomerates, more effective from the point of view of plant protection, such as quinones, occurred [30]. Moreover, in seedlings treated with the maximum concentration of the pathogen, the process of biochemical transformation of phenols into other structural components took twice as long due to their greater accumulation in plants than in seedlings inoculated with the pathogen at concentrations of $10^2 - 10^4$ CFU/ml. The amount of phenols increased in proportion to the time and the increase in the inoculum concentration. At 96 and 120 hours of the experiment, a slow increase in the amount of phenolic compounds in seedlings of all studied samples was observed in proportion to the increase in the concentration of the fungal inoculum, which ranged from 4.6 to 6.9 mg/g of dry biomass, respectively. At the same time, in the control seedlings of Renan variety at 96 and 120 hours of the experiment, the graphical curve of the amount of accumulated phenolic compounds repeated the pattern of their pathogen-induced accumulation and averaged 4.5 and 6.3 mg/g dry biomass.

The results of experiments of pathogen-induced accumulation of phenols in 8 – 12 days-old seedlings of Renan variety showed that it occurs most intensively on the first day, after which their concentration decreases in wheat plants of this variety, which is probably due to their overoxidation into quinones [23] over the next 1 – 2 days. Studies of the amount of quinone accumulation in the same model can either confirm or refute this assumption. The time interval for the decrease in phenolics concentration in Renan seedlings depended on the concentration of the pathogen inoculum (Fig. 2). The higher the concentration of the pathogen inoculum, the more phenolics were accumulated by plants and the longer the pause in their further growth.

Conclusions

The ability of intact seedlings of Renan variety to accumulate a large amount of constitutional callose and phenolic compounds in the early stages of vegetation was determined, which directly correlates [34] with the resistance of this variety to the pathogen of ocular spot *Pseudocercospora herpotrichoides*.

The intensive accumulation of the studied protective substances in Renan seedlings over time may be a decisive factor of resistance to the influence of the pathogenic fungus *P. herpotrichoides*. That is, repeated inoculation with the pathogen or exposure to other unfavourable environmental factors on Renan plants can initiate a faster response due to the formed superorganised structures.

Thus, it is highly probable that the cooperative [32] reorganisation of biochemical processes in Renan plants and the presence of superorganised structures in them determines their higher information capacity and ensures higher system reliability. That is, dissipative structures (non-gentropic pumps - papillae, etc.) stabilise the entropy flux and ensure the maintenance of a stationary non-equilibrium state [39] at a higher information and energy level. In addition, modern electron microscopic, spectroscopic, and spectrofluorimetric research methods can provide a deeper understanding of the formation processes and functions of dissipative structures.

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