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Changes in the transcription activity of the *Ago1*, *Ago2* and *Dcl2* genes in various wheat species under the influence of infection with the causative agent of Septoria

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Motivation and Aim: The search for effective and environmentally friendly approaches to protecting cultivated plants from pathogens is one of the most pressing areas of plant science, since pathogens often lead to significant losses in crop yields. The discovery and explanation of the phenomenon of RNA interference (RNAi), which is involved in the regulation of the activity of various genes in both the host plant and the pathogen [1], made it possible to evaluate the possibility of its use for practical purposes. In this work, we analyzed the activity of the Ago1, Ago2 and DCl2 genes of the RNAi system in a number of representatives of the genus *Triticum* L.: *T. timopheevii*, *T. monococcum* and the *T. aestivum* variety "Salavat Yulaev" during infection with the pathogenic fungus *S. nodorum* Berk.
Methods and Algorithms: Experiments were carried out on wheat plants of the genus *Triticum* L.: *T. timopheevii* k-58666, *T. monococcum* k-39471 and *T. aestivum* of the moderately susceptible variety "Salavat Yulaev". The experiments were carried out in laboratory conditions, where the plants were grown at room temperature (20–22 °C) in a light area with a 16-hour light period. Before the experiments, wheat seeds were sterilized with potassium permanganate solution and washed several times with sterile water. Next, the seeds were germinated in enamel cuvettes on damp filter paper. The dishes and paper were autoclaved at a temperature of 150–180 °C for 1.5–2 hours. Fully expanded first leaves of 7-day-old seedlings were cut off and placed in Petri dishes on damp cotton wool with the addition of benzimidazole (40 mg/l). In the experiment, we used a highly virulent strain of the phytopathogenic fungus *T. aestivum* against common wheat, *Stagonospora nodorum* – SnB, from the collection of the Institute of Biogeochemistry, UFRC RAS. Infection of seedlings with pycnospores of the fungus *S. nodorum* was carried out by applying a spore suspension at a concentration of 105 spores/ml to a leaf with a micropipette with a volume of 4–5 μl per leaf. Isolation of total RNA to assess the level of transcripts of the studied genes was carried out using the Lyra reagent (Biolabmix, Russia), according to the protocol of the supplier. To synthesize cDNA, a reverse transcription reaction was performed using M-MuLV reverse transcriptase (Synthol, Russia). The transcript copy number for each gene under study was determined by quantitative real-time PCR on a CFX Connect Real-Time System device (Bio-Rad, USA) using the intercalating dye EVA-Green (Synthol, Russia) c using the software "Bio-Rad CFX Maestro 1.1 Version: 4.1.2433.1219" (Bio-Rad, USA) relative housekeeping reference gene: TaRLI (AY059462). Measurements

of transcription activity of the studied genes were carried out in 3 biological and 3 analytical replicates. Statistical processing of real-time PCR data was carried out using the Bio-Rad CFX Maestro 1.1 Version: 4.1.2433.1219 software (Bio-Rad, USA), as well as using the Microsoft Excel program (Microsoft, USA).

Results: During the study, we found that the activity of the studied genes of the RNA interfering system of wheat plants actually changed under the influence of the phytopathogen (Fig. 1).

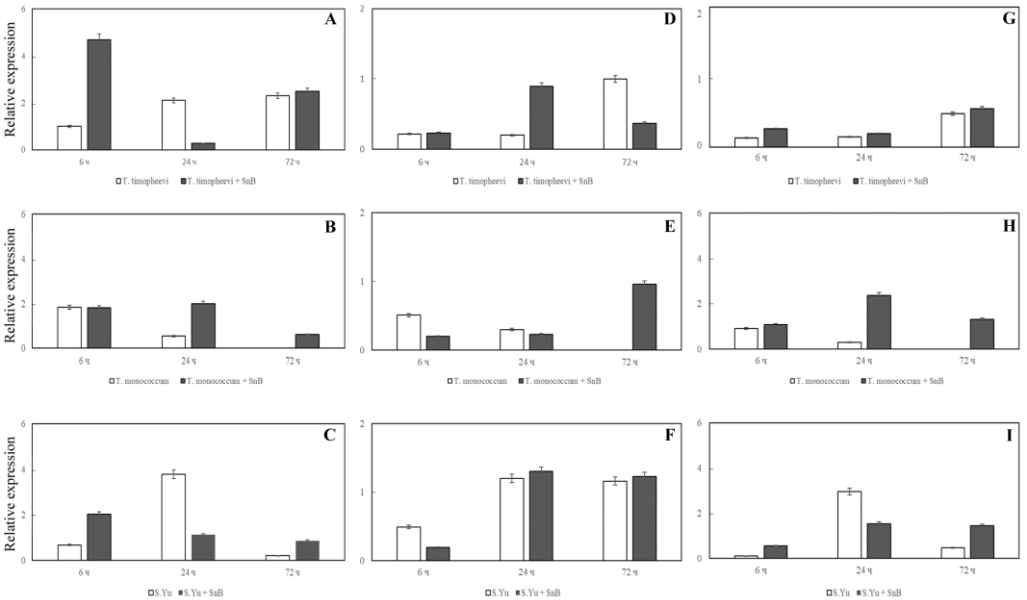

Fig. 1. Changes in transcription activity of the genes Ago1 (A, B, C), Ago2 (D, E, F) and Dcl2 (G, H, I) in *T. timopheevii* (A, D, G), *T. monococcum* (B, E, H) and *T. aestivum* variety "Salavat Yulaev" (C, F, I) under normal conditions and when infected with the pathogenic fungus *S. nodorum* 6, 24 and 72 hours after infection

It was found that in the resistant tetraploid wheat *T. timopheevii*, the activity of the Ago1 gene increased when attacked by a phytopathogen already 6 hours after infection, while the activity of Ago2 was observed only at the 24-hour fixation point, which can be explained by the sequential activation of these Ago genes and correlates with data on their relationship [2]. Similar results were observed when analyzing the studied RNAi genes in the hexaploid bread wheat variety "Salavat Yulaev", which is moderately susceptible to the Septoria pathogen. On the other hand, this variety showed a tendency to activate a gene from the Dcl family, which correlates with data previously obtained in our laboratory [3]. At the same time, the activity of the Ago and Dcl genes increased at later stages of pathogenesis in diploid *T. monococcum*.

Conclusion: Thus, our data indicate the involvement of the protein components Ago and DCL, involved in the phenomenon of RNA interference, in the protective response of wheat plants against the phytopathogen *S. nodorum*.

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