Вавиловский журнал генетики и селекции. 2023;27(8):1000-1009
DOI 10.18699/VJGB-23-115

Original Russian text https://vavilovj-icg.ru/

The effect of salicylic and jasmonic acids on the activity of *SnAGO* genes in the fungus *Stagonospora nodorum* Berk. in *in vitro* culture and during infection of wheat plants

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Abstract. RNA interference is a gene silencing mechanism that plays an important role in genetic regulation in a number of eukaryotes. Argonaute (AGO) proteins are central to the complex RNA interference system. However, their role in this mechanism, both in the host plant organism and in the pathogen, has not yet been fully elucidated. In this work, we identified and phylogenetically analyzed the *SnAGO1*, *SnAGO2*, *SnAGO3*, and *SnAGO18* genes of the pathogenic fungus *Stagonospora nodorum* Berk., and analyzed their expression under conditions of infection of plants with varying degrees of resistance to the pathogen. The expression level against the background of plant immunization with the resistance inducers salicylic and jasmonic acids was assessed. In addition, the activity of these genes in the culture of the fungus *in vitro* was studied under the direct influence of resistance inducers on the mycelium of the fungus. Earlier activation of the *SnAGO* genes in *in vitro* culture under the influence of salicylic and jasmonic acids suggests their sensitivity to it. In an *in vivo* system, plant immunization to induce the accumulation of pathogen *SnAGO* transcripts was found. At the same time, the *SnAGO* genes of the fungus *S. nodorum*, when interacting with plant cells, reacted depending on the degree of host resistance: the highest level of transcripts in the resistant variety was observed. Thus, our data prove that the *SnAGO* genes of the fungus *S. nodorum* effectively interact with the host defense system in direct proportion to the degree of resistance of the latter to the pathogen. It was proposed to use the ratio of the transcriptional activity of the fungal reference gene *SnTub* to the host *TaRLI* gene as a marker of disease development in the initial period of the infectious process.
Key words: RNA interference; *SnAGO* genes; fungus *Stagonospora nodorum*; common wheat; pathogenesis, salicylic acid; jasmonic acid.

For citation: Shein M.Yu., Burkhanova G.F., Maksimov I.V. The effect of salicylic and jasmonic acids on the activity of *SnAGO* genes in the fungus *Stagonospora nodorum* Berk. in *in vitro* culture and during infection of wheat plants. *Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding*. 2023;27(8):1000-1009. DOI 10.18699/VJGB-23-115

Влияние салициловой и жасмоновой кислот на активность генов *SnAGO* гриба *Stagonospora nodorum* Berk. в культуре и при инфицировании растений пшеницы

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Аннотация. РНК-интерференция представляет собой механизм подавления генов, играющий важную роль в генетической регуляции у эукариот. Белки Argonaute (AGO) занимают центральное место в сложной системе явления РНК-интерференции. Однако их роль в этом механизме, как в организме растения-хозяина, так и у патогена, до сих пор полностью не исследована. Мы провели идентификацию и филогенетический анализ генов *SnAGO1*, *SnAGO2*, *SnAGO3* и *SnAGO18* патогенного гриба *Stagonospora nodorum* Berk., возбудителя септориоза пшеницы, и проанализировали их экспрессию в условиях инфицирования растений с различной степенью устойчивости к патогену. Уровень экспрессии оценивали на фоне иммунизации растений индукторами устойчивости: салициловой и жасмоновой кислотами. Также изучена активность указанных генов в культуре гриба при непосредственном воздействии индукторов устойчивости на мицелий гриба. Выявленная более ранняя активация генов *SnAGO* в культуре под влиянием салициловой и жасмоновой кислот указывает на их чувствительность к ним. В системе *in vivo* обнаружено, что иммунизация растений индуцирует накопление транскриптов *SnAGO* патогена. При этом гены *SnAGO* гриба *S. nodorum* при взаимодействии с растительными

клетками реагировали в зависимости от степени устойчивости хозяина: наиболее высокий уровень транскриптов наблюдался в устойчивом сорте. Таким образом, полученные данные доказывают, что гены *SnAGO* гриба *S. nodorum* эффективно взаимодействуют с системой защиты хозяина в прямой зависимости от степени устойчивости последнего к патогену. Предложено использовать отношение транскрипционной активности грибного референсного гена *SnTub* к хозяйскому гену *TaRLI* в качестве маркера развития болезни в начальный период инфекционного процесса.
Ключевые слова: РНК-интерференция; гены *SnAGO*; гриб *Stagonospora nodorum*; мягкая пшеница; патогенез; салициловая кислота; жасмоновая кислота.

Introduction

Phytopathogenic fungi are a powerful risk to food security, which limits the biological potential of agricultural plants and reduces the quality of the resulting products. At the present stage, methods of plant protection are being developed based on natural systemic and cellular phytoimmunity, where a special place is occupied by a unique mechanism that disables gene expression, described by the term "RNA interference" (RNAi) – an evolutionarily conservative and at the same time highly specific immune component for almost all eukaryotes.

Methodologically, when creating modern approaches to plant protection, it is necessary to take into account that during the interaction of plants with pathogens, especially fungal ones, the RNAi components of not only the host, but also the pathogen are activated. Induction of the activity of a number of genes responsible for the functioning of RNAi in pathogenic fungi suggests the possibility of their participation in the suppression of genes of the host defense system (Weiberg et al., 2013). It should be noted that the role of the RNAi mechanism in the evolution and life of fungi is important and can vary quite significantly depending on the survival strategy, the method of infection and spread, as well as the pathogens that infect the fungus itself (Neupane et al., 2019). For example, it is known that artificial inactivation of one or two genes encoding RNAi proteins in phytopathogenic fungi disrupts virulence (Raman et al., 2017; Wang et al., 2018).

Argonaute proteins (AGOs) are considered key components in the complex RNAi phenomenon and bind short microRNAs (Feng et al., 2017; Neupane et al., 2019). The most important function of AGO proteins, actively discussed in the scientific literature, is participation in phytoimmunity. For example, we previously observed that pre-treatment of seeds with salicylic acid (SA) formed the resistance of wheat to Septoria nodorum blotch (SNB), and at the same time, active accumulation of *TaAGO1* gene transcripts was observed in plant tissues infected with the causative agent of this disease (Shein et al., 2021). So, in tobacco plants *Nicotiana attenuata* Torr. ex S.Watson, the accumulation of the *NaAGO4* protein turned out to be critical in the formation of resistance to the fungus *Fusarium brachygibbosum* Padwick (1945) through the jasmonate signaling pathway (Pradhan et al., 2020). Disruption of this process turned off the synthesis of jasmonic acid (JA) in plants and led to their infection, but resistance to the fungus was restored after treating the plants with JA. It can be assumed that the functioning of the plant defense system, regulated by JA, mediates the operation of the mechanism of the RNAi phenomenon. The importance of plant proteins AGO18 in the formation of antiviral defense in rice was discovered (Yang et al., 2020).

It is also known that AGO proteins are actively involved in physiological processes occurring in the mycelium of various types of fungi. Thus, genes encoding AGO proteins were identified in the genome of the fungi *Fusarium graminearum* (*FgAGO1*) (Chen et al., 2015) and *Metarhizium robertsii* (*MrAGO1*) (Meng et al., 2017). The participation of fungal proteins of the AGO family in the formation of compatibility between the host and the pathogen was demonstrated in the fungi *Verticillium dahlia* and *V. longisporum* (Shen et al., 2014). A similar effect was observed in the fungus *Scletotinia sclerotiorum*: mutants of the *AGO2* gene of this fungus had slow growth and reduced virulence (Neupane et al., 2019). Suppression of *AGO2* (*QDE-2*) gene expression also reduced virulence in the fungi *Valsa mali* (Feng et al., 2017) and *Fusarium oxysporum* f. sp. lycopersici (Jo et al., 2018).

Thus, proteins of the AGO family are key components in the functioning of not only plant but also fungal defense systems mediated by RNAi mechanisms. At the same time, it should be noted that so far little work has been devoted to the analysis of the expression of genes encoding proteins of the RNAi mechanism in various pathogens under conditions of plant infection and preliminary immunization with phytohormones SA and JA. In this work, such an analysis was carried out using a model of the phytopathogenic fungus *Stagonospora nodorum* Berk. (*Septoria*, *Parastagonospora*, *Phaeosphaeria*), which causes SNB – one of the most harmful wheat diseases. The main objective was to evaluate changes in the transcriptional activity of the *SNAGO* genes encoding AGO proteins in an *in vitro* culture of the fungus and under conditions of infection of plants with this fungus, contrasting in resistance to SNB against the background of SA and JA treatment.

Materials and methods

Object. In the experiment, we used a highly virulent strain of the phytopathogenic fungus *S. nodorum* SnB against common wheat *Triticum aestivum* L. (Zhnitsa cultivar) from the collection of the Institute of Biochemistry and Genetics – Subdivision of the Ufa Federal Research Centre of the Russian Academy of Sciences. As plant material, we used two cultivars of spring bread wheat *T. aestivum* (BAD, $2n = 42$) contrasting in their resistance to SNB: Zhnitsa (susceptible) and Omskaya 35 (resistant).

Cultivation of the fungus *in vitro*. The fungus was cultivated on a liquid potato-glucose nutrient medium in Petri dishes. To do this, a suspension of fungal spores was added to the nutrient medium at a rate of 10^5 spores/ml and cultivated in a KBW E6 climate chamber (Binder GmbH, Germany) at a temperature of 18 °C for 14 days with periodic 16-hour lighting. Solutions of SA with concentrations of 10^{-4}, 10^{-5} and

10^{-6} M, as well as JA solutions with concentrations of 10^{-5}, 10^{-6} and 10^{-7} M were previously added to the experimental versions of the nutrient media. These concentrations were chosen by us as optimal due to the data previously obtained in our laboratory on the influence of SA and JA on the degree of development of SNB in various wheat cultivars in the pathosystem (Yarullina et al., 2011).

The design of the experiment. Wheat seeds were presoaked for 24 hours in solutions containing 10^{-5} M SA and 10^{-7} M JA, or their composition. Control samples were kept in distilled water for the same period of time. Then, the seedlings in isolated vessels on Hoagland–Arnon nutrient medium were placed in a KBW E6 plant growth chamber (Binder GmbH, Germany), with a 16-hour photoperiod at a temperature of 20/24 ºC (night/day). The noted concentrations of SA and JA were selected as a result of preliminary experiments as the most effective in inducing resistance of wheat plants against *S. nodorum* (Yarullina et al., 2011). Then, sections of leaves of 7-day-old control and experimental wheat seedlings were placed in Petri dishes on damp cotton wool with the addition of benzimidazole (40 mg/L). Some of the leaves were infected with fungal spores by applying 4 μL of the suspension (105 spores/mL), according to the method (Veselova et al., 2021). Leaves inoculated with fungal spores in Petri dishes were placed in a thermostat for 24 hours and then transferred to a KBW E6 plant growth chamber (Binder GmbH, Germany).

Visual assessment of the degree of fungal development on wheat leaves. The development of the fungus *S. nodorum* on leaves was monitored daily. The area under the disease development curve in the variants was determined according to the method proposed by A.A. Marchenkova et al. (1991). Leaf lesion area was measured using ImageJ (https://imagej.nih.gov/ij/download.html).

RNA extraction and study of the relative gene expressions of the *SnAGO* genes. Isolation of total RNA from wheat leaves fixed in liquid nitrogen, as well as mycelium of the fungus *S. nodorum* SnB grown *in vitro*, was carried out using the Lyra reagent according to the protocol of Biolabmix (Russia, https://biolabmix.ru biolabmix.ru). The concentration of nucleic acids was measured using a Thermo Scientific™ NanoDrop™ 1000 spectrophotometer (ND1000WOC) at A260/A280. To synthesize cDNA, a reverse transcription reaction was performed using M-MuLV reverse transcriptase (Syntol, Russia). The nucleotide sequences of the studied *SnAGO* genes of the fungus *S. nodorum* were selected from the FunRNA database (http://funrna.riceblast.snu.ac.kr/, 04.12.2023). Primers for these genes were designed using the Internet programs "Primer-Blast" (https://www.ncbi.nlm.nih.gov/tools/primer-blast, 04.12.2023) and "PrimerQuest Tool" (https:/eu.idtdna.com/Primerquest, 04.12.2023) (the Table). To assess the level of gene transcription, we used the quantitative real-time PCR method on the CFX Connect Real-Time System device (Bio-Rad, USA). SYBR Blue reagent (Syntol, Russia) was used as an intercalating dye. The transcriptional activity of the fungal pathogen was assessed relative to the reference gene *SnTUB*, encoding the fungal tubulin protein (Fraaije et al., 2002). To assess the development of the fungus at the RNA level, we used an analysis of the ratio of transcripts of reference genes: the pathogen *SnTub* (Fraaije et al., 2002) and the host *TaRLI*, encoding a wheat RNase L inhibitor-like protein (Giménez et al., 2011) in experimental plants. According to the works of these authors, the expression of the noted genes of the fungus *S. nodorum* and wheat is not affected by environmental factors.

Primers of *SnAGO* gene loci of the fungus *S. nodorum*

Gene designation	Nucleotide sequence
SnAGO1 homologue of *QDE-2*	F GCAAGTTCGCCATGAACAATAA
	R CAAACCTTCTGGACCATCTCTC
SnAGO2	F GGAGACTCACAGTTCGAAGAAG
	R TAGGAGAGGCAGAGGTTGTAA
SnAGO3	F CGTTTCCTGGGTTGACATAGAT
	R GCCAGACGTTCACTCTGATATT
SnAGO18	F GTCAGTCGATCAAGGTGGATTTA
	R CGTATAGTGCTGACGTCTCTTG
SnTub(β)	F TGGTATGGGTACGCTTTTGATCTC
	R GTAGCGACCGTTGCGGAAGTCAGA
TaRLI(a)	F TTGAGCAACTCATGGACCAG
	R GCTTTCCAAGGCACAAACAT

Bioinformatic and statistical analyzes. The experiments were carried out in triplicate. Mean values with standard errors (± SE) are shown in the figures. Statistical analysis of the obtained data was carried out in the Bio-Rad CFX Maestro 1.1 Version: 4.1.2433.1219 program (Bio-Rad, USA). Differences in the studied parameters between individual treatments were analyzed using analysis of variance. Alignment of nucleotide sequences and construction of a phylogenetic tree were carried out using the MEGA11: Molecular Evolutionary Genetics Analysis version 11.0.13 program (Tamura et al., 2021). The MUSCLE algorithm was used for alignment; phylogenetic trees were constructed using the Maximum Likelihood method (Tamura et al., 2021).

Results

Based on previous studies, we selected bread wheat cultivars that contrast in their resistance to the fungus *S. nodorum* (Veselova et al., 2021). Previously, the effect of treating wheat seeds with SA and JA on the subsequent formation of resistance to the fungus *S. nodorum* in seedlings was also assessed (Yarullina et al., 2011). These works showed that SA and JA could significantly reduce the severity of the development of SNB in wheat.

In this work, at the first stage, we analyzed the degree of fungal development in plant leaf tissues under normal conditions and under conditions of inducing the plant defense system using SA and JA pretreatment. The intensity of the formation of an infectious spot and the rapid development of necrosis in the leaves of the Zhnitsa cultivar showed a high degree of susceptibility of this cultivar to the pathogen strain we used (Fig. 1, *a*). The disease symptoms began to appear in the form of brown spots already on the 4th day after

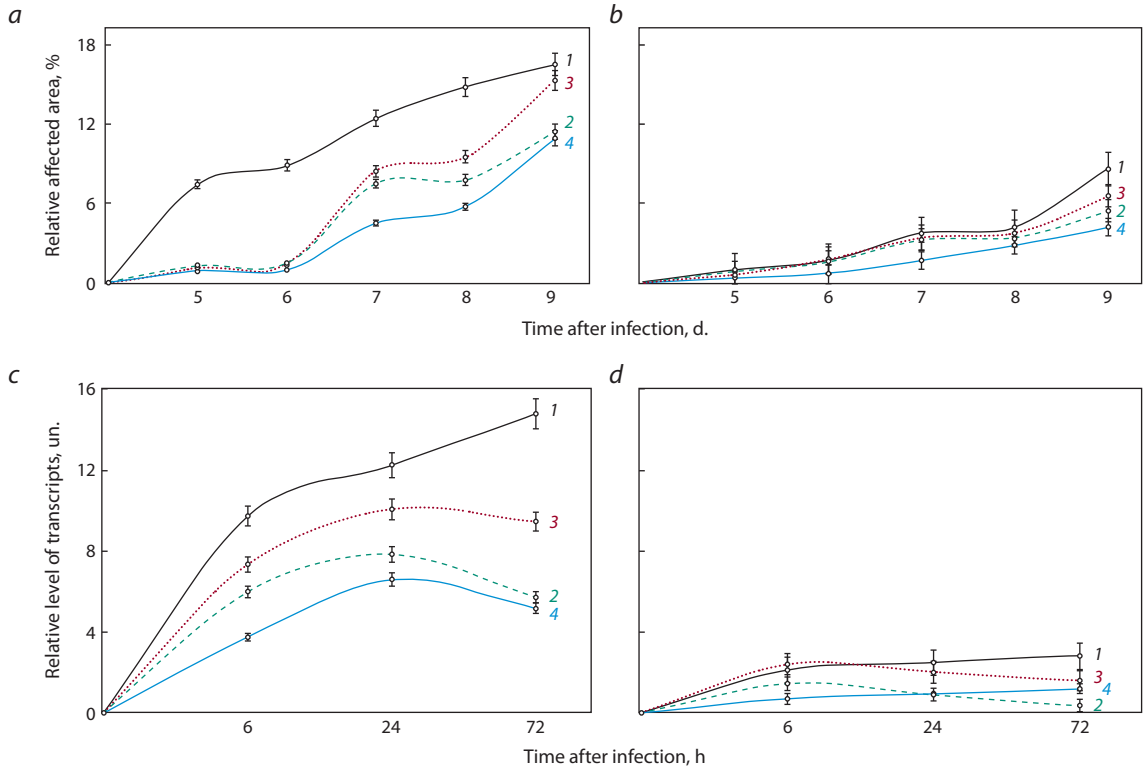

Fig. 1. Comparative changes in leaf damage area (*a*, *b*) and the level of transcripts of the fungal reference *SnTub* gene in relation to the wheat *TaRLI* gene (*c*, *d*) in the leaves of susceptible (*a*, *c*) and resistant (*b*, *d*) cultivars of common wheat in the control (*1*) and after pre-treatment with salicylic (*2*), jasmonic (*3*) acids and their composition (*4*).

inoculation of the leaves, and on the 7th day after infection, the leaves were significantly affected. Under the same conditions, on the resistant cultivar Omskaya 35, SNB developed less intensively (see Fig. 1, *b*, *d*). Accordingly, during the experiment, we confirmed the degree of susceptibility of the studied cultivars in terms of resistance to the fungus *S. nodorum*. In the variant of pre-treatment of seeds with SA and JA, as previously found (Yarullina et al., 2011), inhibition of the development of SNB on leaves infected with the fungus was observed, which suggests a systemic immunizing effect of these compounds. We found a particularly good protective effect in the variant of pre-sowing treatment of wheat seeds with a composition of SA and JA.

It is obvious that the successful development of the fungus *S. nodorum* in plant tissues is accompanied by the accumulation of its biomass and, accordingly, a change in the ratio of proteins and nucleic acids between the host and the pathogen. Based on this, we analyzed the ratio of transcript levels of reference genes: the pathogen *SnTub* and the host *TaRLI* in experimental plants. As can be seen from the data obtained (see Fig. 1, *c*, *d*), the ratio of cDNA of the *SnTub* gene to the *TaRLI* gene in the susceptible Zhnitsa cultivar during the observed period was much higher than in the resistant one. This ratio noticeably decreased in plants pre-treated with SA and JA, as well as their composition (most noticeable after treatment of seeds with SA and JA).

In the resistant cultivars, the most pronounced decrease in the *SnTub*/*TaRLI* ratio was observed after treatment with SA. These data indicate the important contribution of the salicylate-induced pathway in the formation of resistance of wheat plants against the *S. nodorum* pathogen. Accordingly, we can say that the *SnTub*/*TaRLI* indicator is a convenient marker for early rapid diagnosis of resistance of wheat plants to the pathogenic fungus *S. nodorum*, as well as for assessing changes in this resistance at different stages of the formation of the relationship between the host plant and the specified pathogen.

To identify *SnAGO* genes encoding AGO family proteins in the *S. nodorum* genome, an analysis of the FunRNA database (Choi et al., 2014) was carried out using the annotated *S. nodorum* genome sequence (Hane et al., 2007). This approach allowed us to identify the SNOG_12157 locus. The phylogenetic tree of *AGO* genes is presented in Figure 2. As can be seen, the structure of the same *AGO* genes in different organisms has greater homology than the structure of different genes of a given family in representatives of the same genus/taxon. Thus, the *AGO1* gene we selected for analysis in the pathogen *S. nodorum* turned out to be on the same branch as the *AGO1* genes of fungi from other genera or even divisions, and not with the *AGO2*, *AGO3* and *AGO18* genes of the same species. It is also noteworthy that, according to the obtained result, the *Qde2* genes, being homologues of *AGO1* (Jo et al., 2018), are located on a completely different phylogenetic branch of fungal genes. This classification of *AGO1* into a separate group from *AGO2* and *AGO3* correlates with similar results obtained in other studies (Zhang et al., 2015; Ahmed et al., 2021).

Subsequent BlastP sequence analysis identified multiple genes putatively encoding fungal *SnAGOs* based on matches

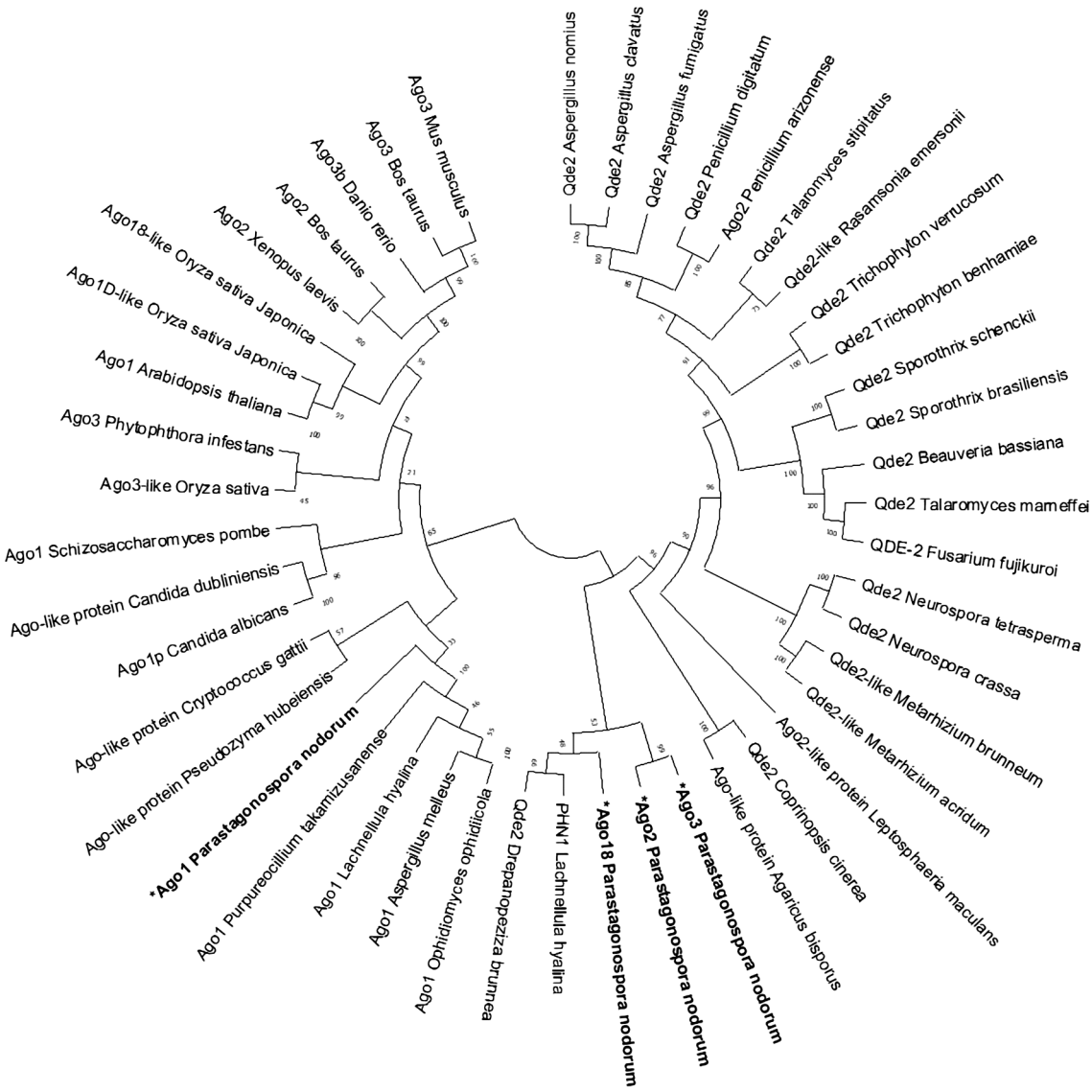

Fig. 2. Phylogenetic tree of the *AGO* and *QDE* genes in various organisms. Sequences of fungi studied in this work are highlighted in bold.

to known motifs characteristic of *AGO* genes. On this basis, the *SnAGO* genes were named *SnAGO1*, *SnAGO2*, *SnAGO3*, and *SnAGO18*, respectively, and were selected for further analysis of transcription activity. Primers for assessing the expression of the *SnAGO1*, *SnAGO2*, *SnAGO3* and *SnAGO18* genes are presented in the Table.

Previously, it was not known how the transcriptional activity of fungal genes responsible for the formation of the RNAi phenomenon changes under conditions of infection of susceptible and resistant wheat cultivars. To do this, we analyzed the level of *SnAGO* gene transcripts identified in the fungus *S. nodorum* under infection conditions in bread wheat cultivars contrasting in resistance to this pathogen, pretreated with SA and JA, as well as with the SA + JA composite (Fig. 3). As can be seen, when wheat leaves are infected with fungal spores during the experiment, transcripts of the *SnAGO1*, *SnAGO2* and *SnAGO3* genes accumulate. Moreover, it is noteworthy that on the resistant cultivar Omskaya 35, the level of gene transcripts increases during the experiment.

Treatment of seeds with SA and JA, as well as their composition, was accompanied by locus-specific changes in the levels of transcripts of fungal *SnAGO* genes in a pathogenic system with both resistant and susceptible wheat cultivars (see Fig. 3). Most loci showed transcript accumulation; however, the extent of accumulation varied depending on the locus and the treatment. For example, the *SnAGO1*, *SnAGO2* and *SnAGO18* genes on the resistant cultivar Omskaya 35 as a result of seed treatment with JA were expressed to a greater extent than under the influence of SA. The composition of SA and JA reduced the activity of the *SnAGO2* and *SnAGO3* genes more pronouncedly in comparison with untreated and infected samples.

In fungus-infected leaf tissues of a resistant cultivar, pretreated with both SA, JA, and their composition, after 24 hours of the experiment, the level of transcription of all studied genes (mostly *SnAGO1*) was higher compared to untreated plants after the same time of infection, but after 72 hours, it was lower than the corresponding levels in untreated plants

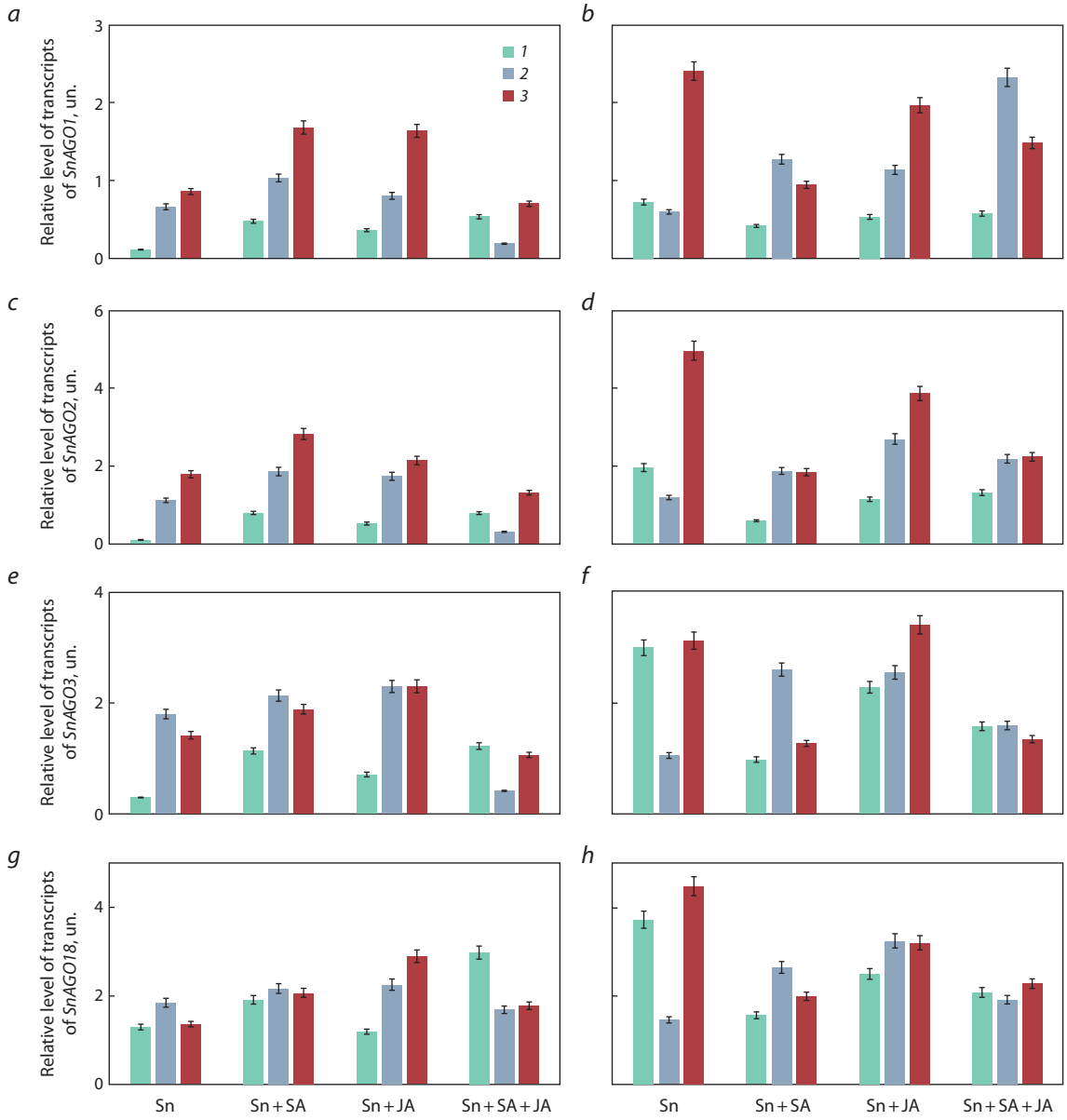

Fig. 3. Changes in the level of transcripts of genes of the *AGO* family in the fungus *S. nodorum* in the leaves of susceptible (Zhnitsa) (*a*, *c*, *e*, *g*) and resistant (Omskaya 35) (*b*, *d*, *f*, *h*) wheat grown from salicylic (SA) and jasmonic (JA) acids treated seeds in 6 (*1*), 24 (*2*) and 72 (*3*) hours after infection.

a, *b*, *SnAGO1* (Snog_12157); *c*, *d*, *SnAGO2* (Snog_10544); *e*, *f*, *SnAGO3* (Snog_10546); *g*, *h*, *SnAGO18* (Snog_12309).

(with the exception of *SnAGO3* after JA treatment). In susceptible plants, throughout the experiment, the expression of the *SnAGO1* and *SnAGO3* genes increased approximately equally under the influence of both SA and JA; separately, the expression of the following genes increased to the greatest extent: *SnAGO2* under the influence of SA and the *SnAGO18* gene under the influence of JA, compared with control infected plants. When wheat seeds were treated with a mixture of SA and JA, the expression level of all *SnAGO* genes in the susceptible cultivar increased after 6 hours of infection (3-fold in the case of *SnAGO18*).

Thus, the obtained results showed that the *SnAGO* genes, encoding one of the key enzymes of the RNAi mechanism, in fungal cells, when interacting with plant cells, respond to the degree of host resistance and increase their transcriptional activity in resistant cultivars. Accordingly, the higher level of *SnAGO* transcripts in pathogen-infected wheat leaves confirms previous suggestions about the important role of this group of proteins in the formation of compatible relationships between wheat and the fungus *S. nodorum* (Shen et al., 2014).

From our point of view, it is interesting to evaluate the direct response of the fungal genome to the effects of SA and JA on a nutrient medium. Thus, using mycelium of the fungus *S. nodorum* grown in liquid culture, we assessed the expression status of *SnAGO* genes when SA and JA solutions of various concentrations were added to the medium on days 5, 7, and 14 after planting on the medium (Fig. 4). It was found that when the fungus was cultivated on a nutrient medium, the activity

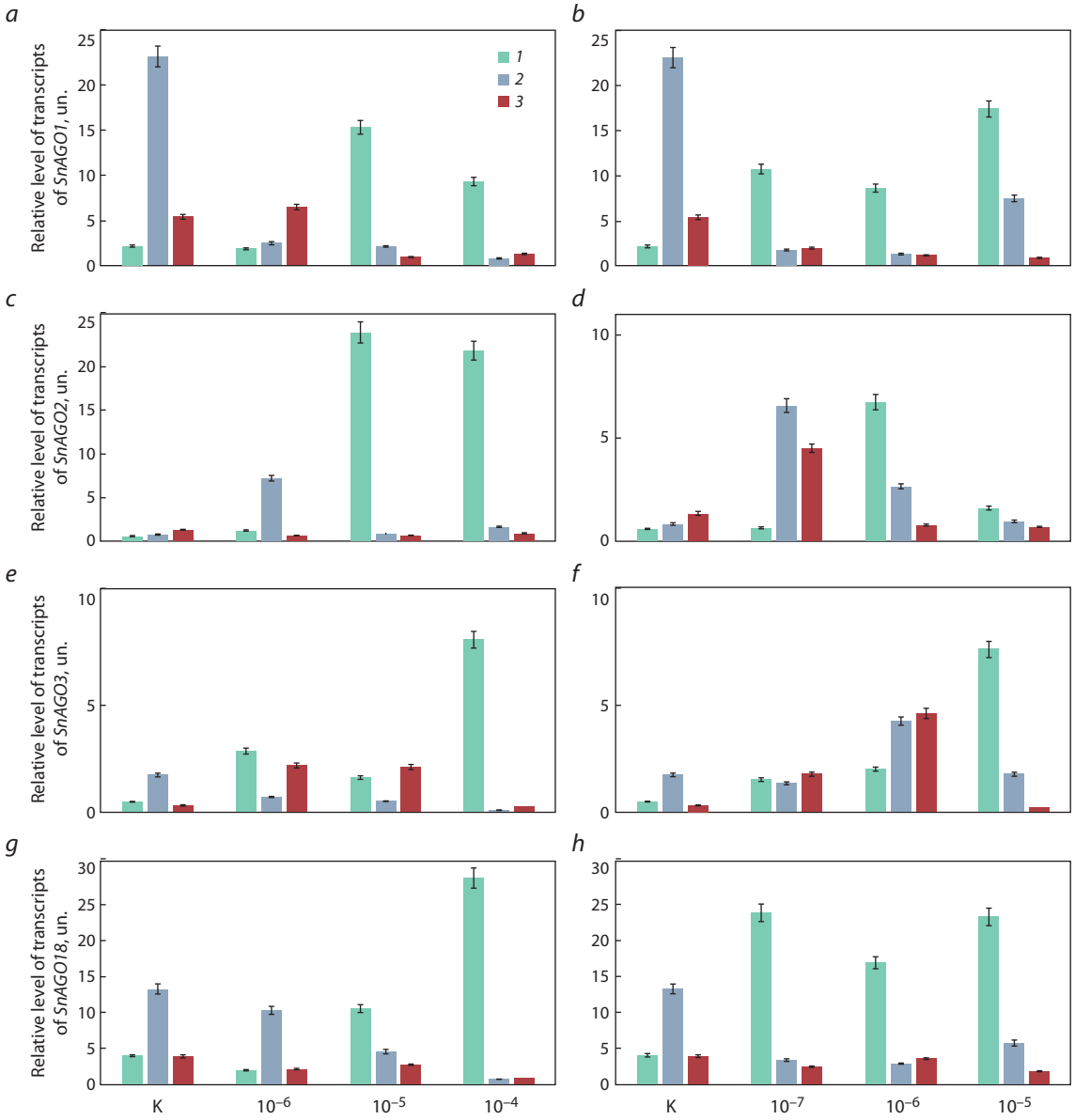

Fig. 4. Changes in the level of transcripts of the *SnAGO* genes of *S. nodorum* grown on a liquid nutrient medium with the addition of salicylic (SA) (*a*, *c*, *e*, *g*) and jasmonic (JA) (*b*, *d*, *f*, *h*) acids of various concentrations (M) in 5 (*1*), 7 (*2*) and 14 (*3*) days after planting.
a, *b*, *SnAGO1* (Snog_12157); *c*, *d*, *SnAGO2* (Snog_10544); *e*, *f*, *SnAGO3* (Snog_10546); *g*, *h*, *SnAGO18* (Snog_12309).

of the *SnAGO1* gene in control samples increased on the 7th day after the start of cultivation. The activity of the *SnAGO2* gene in control colonies of the fungus increased during the experiment, but the effect was less pronounced. When SA was added to the nutrient medium, a pronounced increase in the level of transcripts of all the studied *SnAGO* genes was observed already on the 5th day, and the degree of accumulation of transcripts of these genes was directly proportional to the concentration of the added substance. Similar data were obtained when JA was added to the medium. At the same time, it is noteworthy that the addition of signaling molecules at all JA concentrations and relatively high SA concentrations shifts the accumulation of transcripts to the earliest period – 5 days. Particular attention should be paid to the fact that the *SnAGO1* and *SnAGO18* genes turned out to be the most sensitive to the addition of JA to the nutrient medium.

Discussion

Assessing the mechanisms of formation of plant resistance, especially against pathogens, is an urgent task, the solution of which will make it possible to effectively regulate it and achieve higher productive properties. The diversity of feeding methods of phytopathogenic fungi contributed to the evolutionary formation of various ways of protecting plants from them (McCombe et al., 2022). For example, against biotrophic pathogens that feed on living plant tissues, defense mechanisms are launched, where the SA-dependent signaling pathway plays an active role, forming systemic acquired resistance. JA triggers another signaling pathway, called systemic induced resistance, which protects plants from necrotrophic pathogens and insects. Numerous studies have shown that the causative agent of SNB is characterized by a hemibiotrophic mode of nutrition on wheat plants, combining

a biotrophic phase of development at the very beginning of pathogenesis with a subsequent necrotrophic one; however, it is also capable of saprotrophic growth on nutrient media (Oliver et al., 2012). Accordingly, depending on the timing of pathogen development in plant tissues, host defense systems must clearly recognize these transition stages.

We have previously shown that the highly virulent strain of the pathogenic fungus *S. nodorum* SnB can overcome the defense of wheat associated with the pro-/antioxidant system, including the activation of catalases (Troshina et al., 2010) and chitin deacetylases (Maksimov et al., 2011), and also the accumulation of various effector molecules (Veselova et al., 2021). In this work, we analyzed a number of *S. nodorum* genes encoding *SnAGO* nuclease proteins involved in the RNAi mechanism, using it as a model object.

The RNAi phenomenon represents a unique and ancient mechanism for protecting the genome of eukaryotes, including fungi, from foreign genetic information, and also serves to regulate physiological processes. The most important key components of this complex immune mechanism are AGO RNA nucleases, which have been functionally characterized in model organisms (Choi et al., 2014). It has been shown that the RNAi mechanism in the eukaryotic immune system during the interaction of hosts with their parasites is a "double-edged instrument", which, on the one hand, protects the host from the pathogen, and on the other hand, helps the pathogen disable the accumulation of the most essential key protective proteins of the host, in order to use plant resources for its own functioning. However, to date, the mechanisms of RNAi operation in fungal systems, especially during the development of diseases, have not been sufficiently studied, which limits our understanding of this mechanism.

For example, the formation of compatibility between the host and the pathogen with the participation of plant proteins AGO1 of *Brassica napus* was shown under conditions of plant infection by the fungi *V. dahliae* and *V. longisporum* (Shen et al., 2014). In tomato and *Arabidopsis* plants, microRNAs secreted by the fungus *Botrytis cinerea* used the host AGO1 proteins to selectively suppress the translation of host mitogen-activated protein kinases (MAPKs), peroxiredoxin, and cell wall-associated kinase, and suppression of the accumulation of this protein in mutant *Arabidopsis* plants resulted in reduced susceptibility to the fungus (Weiberg et al., 2013). F. Dunker and co-workers (2020) showed on *Arabidopsis* plants that similar recruitment of the host protein complex AGO1 is also characteristic of the oomycete *Hyaloperonospora arabidopsidis*, a pathogen phylogenetically distant from fungi, suggesting a property common to pathogens.

An important role of the RNAi mechanism in the growth and development of fungi has been revealed, which is naturally reflected in their ability to infect plants. Thus, Δago1 mutants of the fungus *Colletotrichum higginsianum* showed severe defects in conidial morphology (Campo et al., 2016). Deletion of the *FgAGO2* gene of the fungus *Fusarium graminearum* did not affect the fungal phenotype during the asexual phase (Chen et al., 2015), but this gene was found to be important during the fungal sporulation and ascospore maturation stages (Zeng et al., 2018). In another species of fungus, *Fusarium oxysporum* f. sp. *lycopersici* strain 4287, mutants with suppressed expression of the *FoQDE-2* (*AGO1*) gene (Jo et al., 2018) exhibited reduced virulence against tomato plants.

At the moment, there are practically no works that would discuss the behavior of fungal pathogen genes encoding proteins responsible for RNAi under conditions of direct exposure to biostimulants and resistance inducers, for example, SA and JA, or indirect exposure through plant infection. There is conflicting information about the role of plant resistance inducers in the operation of the RNAi mechanism in plant tissues under the direct influence of SA and JA and in response to infection by pathogens. For example, some authors show that SA-induced pathogen resistance affects plant RNAi mechanisms only through the host RNA-dependent RNA polymerase (RDR1) and is coordinated by this protein (Lee et al., 2016). At the same time, there is information that in plants, for example, rice, the expression levels of the *OsAGO1a*, *OsAGO2* and *OsAGO18* genes turned out to be associated with JA, and in mutant rice plants coi1-13, unable to transmit JA signals, they were lower than in wild plants (Yang et al., 2020). This is especially interesting due to the fact that in the pathogenic wheat fungus *S. nodorum*, the role of RNAi in pathogenicity still remains unresolved. At the same time, the participation of RNAi in the development of pathogenicity of *Zymoseptoria tritici*, another fungus that causes septoria leaf blight on wheat, was recently analyzed after knockout of the *AGO1* and *AGO2* genes in its genome (Kettles et al., 2019; Ma et al., 2020). The analysis did not reveal qualitative phenotypic changes in the development of SNB symptoms on the susceptible wheat cultivar Bobwhite. Fungal strains mutant for the *AGO* genes did not lose their virulence properties against wheat plants. From the results obtained, the authors conclude that these proteins do not play a significant role in the development of SNB. At the same time, analysis of the expression status of genes responsible for the operation of the RNAi mechanism in the pathogenic fungus *V. nonalfalfae*, which causes verticillium wilt in hops (*Humulus lupulus* L.), showed that more virulent strains of the pathogen have a higher level of accumulation of *VnAGO* transcripts (Jeseničnik et al., 2019).

We showed that in the pathogen-resistant to *S. nodorum* wheat cultivar Omskaya 35, the accumulation of transcripts of the studied *SnAGO* genes during fungal pathogenesis in leaf tissues was more active than in the susceptible cultivar, which suggests the involvement of the studied genes in the process of overcoming the host's defense system and the possibility of regulating activity of these genes depending on the degree of host resistance. These assumptions are further confirmed in experimental variants where plants were immunized by pre-sowing seed treatment with SA and JA, as well as their composition. As can be seen, the level of transcripts of pathogenic *SnAGO* genes in these variants also turns out to be higher than in the control ones, although, judging by the ratio of housekeeping genes *SnTub*/*TaRLI*, the content of the *SnTub* gene in plants immunized with SA and JA is significantly lower.

We obtained interesting results when assessing the transcriptional activity of *SnAGO* genes after direct exposure of the fungal mycelium in culture to SA and JA. High concentrations of SA enhanced the accumulation of transcripts of the studied *SnAGO* genes at earlier times of cultivation, while low

concentrations did not produce this effect. JA stimulated the accumulation of *SnAGO1*, *SnAGO3*, *SnAGO18* transcripts at early stages of cultivation and at lower concentrations compared to SA. It can be assumed that fungal *SnAGO* genes are sensitive to the direct effects on the fungal mycelium of these signaling molecules, which are positioned as inducers of plant defense systems against pathogens. Thus, we have shown that the fungal RNAi system actively responds to the addition of SA and JA to the cultivation medium, and also participates in the process of plant infection. At the same time, artificial stimulation of the protective properties of the plant also triggers the accumulation of *SnAGO* transcripts of the pathogen. Our data suggest that the *AGO* genes involved in the RNAi system of the fungus *S. nodorum* strain SnB interact effectively with the infecting plant and, most likely, this interaction depends on the degree of host resistance.

The possibility of assessing the degree of development of pathogens in plant tissues using molecular biological methods that assess the level of accumulation of pathogen nucleic acids in tissues is very important, since visual assessment of disease symptoms is often subjective and influenced by various factors. This work uses the *SnTub*/*TaRLI* method for assessing the accumulation of pathogen genetic material in plant tissues by comparing the ratio of transcript levels of fungal and wheat housekeeping genes. Estimation of the *SnTub*/*TaRLI* transcript ratio correlated with the results of visual observation of disease development on leaves. The results also showed that the most effective in inducing the protective properties of wheat plants against SNB was pre-sowing treatment of seeds with JA, as well as with a composition of JA and SA (see Fig. 1). Using the level of the *SnTub*/*TaRLI* ratio, we confirmed the dynamics of accumulation of biological material of the pathogenic fungus, but at earlier periods of observation. In addition, we demonstrated the possibility of using the data obtained on the transcriptional activity of the *AGO* genes of the fungus *S. nodorum* strain SnB as an objective indicator of the activation of its RNAi system.

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Acknowledgements. This work was supported by the Russian Foundation for Basic Research, project No. 20-34-90004.

Conflict of interest. The authors declare no conflict of interest.

Received April 14, 2023. Revised September 19, 2023. Accepted September 25, 2023.