Effect of the host-specific toxin SnTOX3 from Stagonospora nodorum on ethylene signaling pathway regulation and redox-state in common wheat

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The fungus Stagonospora nodorum Berk. is the causative agent of Septoria nodorum blotch (SNB) of wheat. The most important factors of Stagonospora nodorum virulence include numerous fungal necrotrophic effectors (NEs) encoded by SnTox genes. They interact with the matching products of host susceptibility genes (Snn). SnTox-Snn interactions are mirror images of classical gene-for-gene interactions and lead to the development of disease. We have studied the SnTox3-Snn3 interaction, resulting in the development of infection on leaves and formation of extensive lesions. The mechanism of SnTox3 action is likely to be linked to the regulation of redox metabolism and the influence on ethylene synthesis in the wheat plants, although the molecular mechanisms are not fully unveiled. To characterize the SnTox3-Snn3 interaction, we used S. nodorum isolates differing in the expression of the NEs genes SnTox3 (SnB (Tox3+), Sn4VD (Tox3–)) and two soft spring wheat (Triticum aestivum L.) cultivars, contrasting in resistance to the SNB agent and differing in the allelic composition of the susceptibility locus Snn3-B1: Kazakhstanskaya 10 (susceptible) and Omskaya 35 (resistant). We carried out a comparative assessment of the transcriptional activity patterns of genes responsible for ethylene biosynthesis (TaACS1, TaACO) and signaling pathway (TaEIL1, TaERF1) by real-time PCR and estimated the redox state of wheat plants infected with different isolates of S. nodorum by spectrometry. The induction of ethylene biosynthesis and signaling has been shown to result from gene-for-gene interaction between Snn3-B1 and SnTox3. The results of plant redox status estimation showed that ethylene inhibited accumulation of hydrogen peroxide in SnTox3-sensitive genotypes by regulating the operation of various pro-/antioxidant enzymes at the transcriptional and posttranslational levels. Our results suggest that NE SnTox3 influences ethylene biosynthesis and signaling, thereby regulating redox metabolism in infected wheat plants as necessary for successful host colonization at the initial phases of infection, which ultimately leads to extensive lesions due to fast pathogen reproduction.

Key words: Stagonospora nodorum; Triticum aestivum; polymerase chain reaction; real-time polymerase chain reaction; necrotrophic effectors; ethylene; redox-metabolism; gene-for-gene interaction; nonspecific resistance.

For citation: Veselova S.V., Burkhanova G.F., Nuzhnaya T.V., Rumyantsev S.D., Maksimov I.V. Effect of the host-specific toxin SnTOX3 from Stagonospora nodorum on ethylene signaling pathway regulation and redox-state in common wheat. Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding. 2019;23(7):856-864. DOI 10.18699/VJ19.559

Влияние хозяин-специфичного токсина SnTOX3 патогена Stagonospora nodorum на сигнальный путь этилена и редокс-статус растений мягкой яровой пшеницы

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Важнейший фактор вирулентности возбудителя септориоза пшеницы Stagonospora nodorum Berk. – многочисленные некротропные эффекторы (НЭ) гриба (SnTox), взаимодействующие с продуктами генов восприимчивости хозяина (Snn). Взаимодействия SnTox-Snn осуществляются по типу ген-на-ген и ведут к развитию болезни. В настоящей работе изучено взаимодействие SnTox3-Snn3, результатом которого является развитие инфекции на листьях с образованием обширных зон поражения. Предположительно, механизм действия SnTox3 связан с регуляцией редокс-метаболизма и влиянием на синтез этилена у растений пшеницы, однако молекулярные механизмы до конца не раскрыты. Для характеристики взаимодействия SnTox3-Snn3 в работе были использованы изоляты S. nodorum, различающиеся по экспрессии гена SnTox: SnB (Tox3+) и Sn4VD (Tox3–), и два сорта мягкой яровой пшеницы (Triticum aestivum L.), контрастные по устойчивости к возбудителю септориоза и различающиеся по апелльному составу локуса восприимчивости Snn3-B1: Казахстанская 10 (восприимчи-
Introduction

Wheat, a staple crop, has been attacked by various kinds of leaf spot diseases in recent decades, and Septoria nodorum blotch (SNB) ranks among the most injurious ones. It is caused by the fungal pathogen *Stagonospora nodorum* Berk. Yield losses inflicted by this pest reach up to 30 % in susceptible wheat cultivars under permissive environmental conditions (Bertucci et al., 2014). Studies of SNB have been intensively conducted over the past three decades, but there is still no clear understanding of the mechanisms that underlie wheat resistance/susceptibility to infection, on the one hand, and pathogen virulence, on the other hand (Fraaie et al., 2002; Bertucci et al., 2014; Winterberg et al., 2014; Phan et al., 2016; Shi et al., 2016).

It has been shown that among the most important factors of virulence of *S. nodorum* are the numerous necrotrophic effectors (NEs), formerly referred to as host-specific (selective) toxins (Phan et al., 2016; McDonald, Solomon, 2018). The interaction in the wheat–*S. nodorum* pathosystem is of the gene-for-gene type (McDonald, Solomon, 2018). These relationships are confirmed by the fact that the products of the pathogen virulence genes (=host-specific toxins) (SnTox) cause compatibility, i. e. disease expansion, when interacting with products of the host plant susceptibility genes (*Snn*). (Phan et al., 2016). The effect of each SnTox-*Snn* interaction is incomplete and is complemented by other interactions. To date, eight SnTox-*Snn* interactions are known, while only three genes encoding NEs (*SnToxA, SnTox1, and SnTox3*) have been cloned from the pathogen, and only two susceptibility genes (*Tsn1* and *Sn1*) have been cloned from wheat (Phan et al., 2016; Shi et al., 2016).

The genetics of the relationship between wheat and *S. nodorum* is very complex, race-specific resistance explaining only about 40 % of phenotypic manifestations (Shi et al., 2016). In addition, it was shown in the last four years that some of the characterized SnTox-*Snn* interactions causing susceptibility are aimed at manipulating nonspecific plant defense pathways associated with redox metabolism, secondary metabolism and pathogenicity-related proteins (Winterberg et al., 2014; Phan et al., 2016; Shi et al., 2016).

The *Sn3-B1-SnTox3* interaction plays a significant role in SNB development (Shi et al., 2016; McDonald, Solomon, 2018). It is assumed that the result of this interaction is the development of infection on leaves with the formation of extensive lesions, which is associated with the influence of SnTox3 on the generation of reactive oxygen species (ROS); unfortunately, the mechanism underlying this effect remains obscure (Winterberg et al., 2014). Nevertheless, a recent study shows that SnTox3 induces methionine accumulation and ethylene synthesis in wheat plants within 24 h after infection (Winterberg et al., 2014).

One of the earliest plant responses to the penetration of a pathogen is known to be local ROS generation, which plays an important role in the development of systemic resistance (Barna et al., 2012). Currently, the mechanisms regulating apoplastic ROS synthesis during immune response are intensively studied but still insufficiently understood. Recent studies have shown that the pro-/antioxidant state of plants is under the strict control of plant hormones involved in the formation of defense reactions during stress (Barna et al., 2012). They include ethylene, whose role in biotic stress is complex and depends on the type of pathogen and plant species (Vleeshauver et al., 2010; Barna et al., 2012). Earlier, we showed the negative role of ethylene in the development of wheat plant resistance to *S. nodorum* (Veseleva et al., 2016). Unfortunately, the mechanisms of action of plant hormones, including ethylene, on ROS generation under biotic stress are poorly known (Barna et al., 2012).

In this regard, the aim of this work was a comparative assessment of the transcriptional pattern of genes involved in ethylene biosynthesis and signaling pathway genes and of the redox state of wheat plants infected with *Stagonospora nodorum* isolates differing in NE SnTox3 expression.

Materials and methods

The objects of the study were two cultivars of soft spring wheat (*Triticum aestivum* L.) contrasting in resistance to *S. nodorum* Berk.: susceptible cv. Kazakhstanskaya 10 (Kaz10) and resistant cv. Omskaya 35 (Oms35). The pathogen objects were two isolates of the fungus *S. nodorum*: Sn4VD (Republic of Belarus) and SnB (Republic of Bashkortostan). Fungi were grown on potato-glucose agar (PGA). Plants were hydroponically grown on 10 % solution of Hoagland–Arnon nutrient medium in a KS-200 SPU growth chamber (Russia).
20/24 °C (night/day) at the irradiance 146 W/m² FAR (Osram lamps L 36W/77) and the 16-h photoperiod for seven days. The assessment of seedling resistance of cultivars was carried out by the labs methods, as described in (Veselova et al., 2016). The resistance/susceptibility of cultivars was assessed from the lesion area seven days after inoculation with S. nodorum isolates. The development of SNB symptoms on wheat leaves was photographed with an SP-800UZ Image Stabilization camera (Olympus, Indonesia). The lesion area was measured with ImageJ program (rsbweb. nhgri.nih.gov/download.html) and expressed as percent leaf area infected. The degree of damage was also assessed according to the International scale based on the percentage of the affected area of plant organs: RR (0–5 %) – cultivars with very high and high resistance; R (up to 10–15 %) – resistant cultivars; M (up to 25 %) – slightly susceptible cultivars; S (up to 40–65 %) – susceptible cultivars; SS (over 65 %) – cultivars with very high and high susceptibility.

DNA was isolated from wheat seedlings and 7-day fungus culture by the phenol-detergent method (Maniatis et al., 1984). SnTox3 gene (FJ823644) identification in S. nodorum isolates was performed by PCR with gene-specific primers (5´→3´): F–CGAGCTGATATCCCGTTTGA; R–GGGACAGTGACAAATAGGTAAGG (Winterberg et al., 2014); primers for the housekeeping gene tubulin (SS6922) (Fraaije et al., 2002) being used as an internal control for the presence of fungal DNA. Analysis of SnTox3 gene expression in different isolates of S. nodorum during inoculation of wheat plants was performed with the same primers using semi-quantitative PCR. Total RNA was isolated with Trizol reagent (Sigma, Germany) according to manufacturer’s recommendations from leaves of susceptible wheat cv. Kaz10 and resistant cv. Om35 fixed in liquid nitrogen after their inoculation with a pathogen. To obtain cDNA based on RNA from the studied samples, reverse transcription reaction was performed using reverse transcriptase in accordance with manufacturer’s protocol (Synthol, Russia). PCR with the cDNA template was performed in a TP4-PCR-01-Tertsik type PCR machine (DNK-Tekhnologia, Russia).

The allelic state of the Snm3-B1 locus was determined by PCR with primers for the Xcf2d0 and Xgwm234 microsatellite markers (Bertucci et al., 2014). The sequences of primers Xcf2d0 (5´→3´): F–TGATGGGAAGGTAAATGGGAG; R–ATCCAGTCTCTGTCCCAAAGC; of primers Xgwm234 (5´→3´): F–GAGTCTGTATGAGCTGTTG; R–CTGATTGGGTTGTTGACGT (Bertucci et al., 2014). In all cases, PCR products were resolved in 7 % PAAG stained with ethidium bromide using the Gene Ruler DNA Ladder (Fermentas). Gels were photographed using a GelDoc XR documenting system (Bio-Rad, USA).

To reveal the effect of SnTox3 on the biosynthesis and signalling pathway of ethylene, part of the wheat seedlings were treated with 1.5 mM solution of ethephon (ET) (2-chloroethylphosphonic acid), an ethylene-releasing compound (Sigma, Germany) (Veselova et al., 2016), 24 h before inoculation with various S. nodorum isolates, while the remainder of the wheat seedlings were treated with 50 μM aminoethoxyvinylglycine (AVG), ethylene biosynthesis inhibitor (Sigma, Germany). After the treatment, the vessels were closed and kept in the dark. The content of hydrogen peroxide (H₂O₂) and the activities of peroxidase enzymes (PO), oxalate oxidase (OXO), and catalase were measured 24 and 72 h after inoculation with S. nodorum isolates as previously described (Veselova et al., 2018).

Total RNA was isolated from control and experimental wheat leaves with Trizol reagent according to manufacturer’s (Sigma, Germany) recommendations. Prior to the isolation, the leaves were fixed in liquid nitrogen 24 h after their inoculation with S. nodorum. Analysis of the expression of genes for oxidoreductases and genes involved in the biosynthesis and signaling pathways of ethylene was performed by quantitative real-time PCR with an iCycler iQ5 Real-Time PCR Detection System (Bio-Rad, USA) and SYBR Green I intercalating dye (Sintol, Russia). To normalize the expression results of the studied genes, primers for the RLR gene for constitutively expressed RNA inhibitor protein (RNase L inhibitor-like) (AY059462) were used (Gimenez et al., 2011). Changes in the expression of the gene of interest were estimated by the level of normalized gene expression calculated with the iCycler iQ5 Real-Time Detection System Software (Bio-Rad, USA). Primers for the genes encoding NADPH oxidase (TaRboh, AY651113) (Giovanini et al., 2006), superoxide dismutase (SOD) (TaSod, JX398977.1) (Giovanini et al., 2006), anionic peroxidase (TaPrx, TC151917) (Maksimov et al., 2014), aminocyclopropane synthase (ACC synthase – TaACS1, U35779) (Subramaniam et al., 1996), aminocyclopropane oxide-dase (ACC oxidase – TaACO, KF900072) (primer sequences (5´→3´): F–TGTCCATCGCCTCCTTCCTA; R–CGAACACGACCTTGGGTAT; transcription factor of the ethylene signaling pathway EIN3-LIKE1 (ETHYLENE INSENSITIVE3-LIKE1) (EIL1) – TaEIL1, Ku030837, Arabidopsis orthologue gene ATEIN3) (Liu et al., 2016) and the transcription factor of the primary response to ethylene ERF1 (ETHYLENE RESPONSE FACTOR1 – TaERF1, EF583940) (Dong et al., 2010) were used in this study.

All experiments were carried out three times with three biological and three analytical replications (n = 9 in total), except for the measurements of infected area, which were performed in not less than 30 biological replications (n = 90 altogether). The Figures 1–3 and Tables 1–3 report mean values and their confidence intervals calculated from their standard errors. Significance of differences between experimental variants was estimated by Student’s t-test at the confidence level p ≤ 0.05.

**Results**

**SnTox3 gene and Snm3-B3 susceptibility locus.** Two S. nodorum isolates, SnB and Sn4VD, were tested for the presence/absence of the SnTox3 gene by PCR. The gene was found in both (Fig. 1, a).

However, analysis of the transcriptional activity of this NE gene showed no expression in the avirulent Sn4VD isolate and accumulation of SnTox3 transcripts after inoculation of the susceptible cv. Kaz10 and the resistant cv. Om35 with the virulent isolate SnB (see Fig. 1, b). PCR diagnostics of the allelic state of the Snm3-B1 locus was performed in two soft spring wheat cultivars, Kaz10 and Om35. For this purpose, specific primers for two Xcf2d0 and Xgwm234 microsatellite markers flanking the Snm3-B1 locus were used (Bertucci et al., 2014; Shi et al., 2016). The null allele was not found in these varieties. However, the cultivars differed in the allelic...
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Table 1. Reaction of two wheat cultivars with different allelic states of the Snm3-B1 locus to inoculation with S. nodorum SnB (Tox3+) and Sn4VD (Tox3–) isolates

| Cultivar/isolate combination | Damage area, % | Group | Cultivar/isolate combination | Damage area, % | Group |
|------------------------------|----------------|-------|------------------------------|----------------|-------|
| Kaz10/SnB                   | 80.0 ± 3.0     | SS    | Om35/SnB                    | 10.9 ± 2.5     | R     |
| Kaz10/SnB+AVG**             | 94.9 ± 2.3     | SS    | Om35/SnB+ET                 | 57.2 ± 2.6     | S     |
| Kaz10/Sn4VD                 | 18.6 ± 2.2     | M     | Om35/SnB+AVG                | 9.1 ± 1.9      | R     |
| Kaz10/Sn4VD+ET              | 8.4 ± 2.2      | R     | Om35/Sn4VD                  | 1.6 ± 0.5      | RR    |
| Kaz10/Sn4VD+AVG             | 8.1 ± 1.8      | R     | Om35/Sn4VD+ET               | 1.9 ± 1.1      | RR    |
| Kaz10/Sn4VD+AVG**           | 6.5 ± 2.6      | R     | Om35/Sn4VD+AVG              | 1.6 ± 0.5      | RR    |

*a RR (0–5 %) – cultivars with very high and high resistance; R (5–15 %) – resistant cultivars; M (15–25 %) – slightly susceptible cultivars; S (25–65 %) – susceptible cultivars; SS (65–100 %) – cultivars with very high and high susceptibility.

** Plants were treated with either ethephon (ET) or ethylene biosynthesis inhibitor (AVG) 24 h before inoculation with S. nodorum.
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The transcriptional analysis of ethylene biosynthesis and signaling pathway genes measured in Kazakhstanskaya 10 and Omskaya 35 wheat cultivars contrasting in SNB resistance 24 h after inoculation with S. nodorum isolates

| Variant of treatment | Gene    | Variant of treatment | Gene    |
|----------------------|---------|----------------------|---------|
|                      | TaACS   |                      | TaACO   |
| Kaz10               | 100     | Kaz10/SnB            | 311 ± 60|
| Kaz10/SnB+AVG       | 143 ± 23| Kaz10/SnB+ET         | 350 ± 34|
| Kaz10/Sn4VD         | 128 ± 28| Kaz10/Sn4VD+AVG      | 117 ± 9 |
| Kaz10/Sn4VD+ET      | 121 ± 10|                      |         |
|                      |         |                      | TaEIL1  |
| Kaz10               | 100     | Kaz10/SnB            | 237 ± 30|
| Kaz10/SnB+AVG       | 77 ± 3  | Kaz10/SnB+ET         | 223 ± 10|
| Kaz10/Sn4VD         | 110 ± 10| Kaz10/Sn4VD+AVG      | 63 ± 15 |
| Kaz10/Sn4VD+ET      | 65 ± 6  |                      |         |
|                      |         |                      | TaERF1  |
| Kaz10               | 100     | Kaz10/SnB            | 1400 ± 200|
| Kaz10/SnB+AVG       | 389 ± 0 | Kaz10/SnB+ET         | 1810 ± 270|
| Kaz10/Sn4VD         | 322 ± 10| Kaz10/Sn4VD+AVG      | 390 ± 60 |
| Kaz10/Sn4VD+ET      | 375 ± 20|                      |         |
|                      |         |                      |         |
| Om35                | 100     | Om35/SnB             | 86 ± 10 |
| Om35/SnB+ET         | 169 ± 8 | Om35/SnB+AVG        | 102 ± 20|
| Om35/Sn4VD         | 97 ± 10 | Om35/Sn4VD+ET        | 125 ± 6 |
| Om35/Sn4VD+AVG      | 108 ± 20|                      |         |
|                      |         |                      |         |
| Control             |         | Sn                   | 47 ± 10 |
| Sn                    |         | Sn+AVG               | 77 ± 6  |
| Sn+ET                |         |                     | 92 ± 10 |
|                      |         | TaRbohF, TaSod, and PO (TaPrx) (Table 3). However, in Tox3-insensitive plants (Om35/SnB+ET cultivar/isolate combination), the suppression reaction of oxidative burst was less pronounced than in other combinations leading to a compatibility reaction (see Fig. 2, 3, Table 3).

The incompatibility reactions in the Om35/SnB, Om35/Sn4VD, Kaz10/Sn4VD, Kaz10/SnB+ET cultivar/isolate combinations and in ET-treated plants from all combinations were characterized by an increase in \( \text{H}_2\text{O}_2 \) generation (see Fig. 2, a, b) due to a decrease or absence of increase in CAT activity, a drastic increase in the activity of PO and OXO (see Fig. 3), and the accumulation of \( \text{TaRbohF} \), \( \text{TaSod} \), and \( \text{TaPrx} \) transcripts at the early stage of infection (24 h) (see Table 3), which led to the development of a hypersensitive-type response and arrest of \( \text{TaEIL1} \) mRNA was low as compared to control plants (see Table 2).

Redox state of infected plants. We studied the components of the pro-/antioxidant system in order to elucidate the role of SnTox3 in the regulation of redox metabolism in the host plant via the biosynthesis and signaling pathway of ethylene in suppressing defense reactions in infected plants. The compatibility reactions in susceptible plants (Kaz10/SnB) and in plants treated with ET (Kaz10/SnB+ET, Om35/SnB+ET) were characterized by a decrease in \( \text{H}_2\text{O}_2 \) content (Fig. 2, a, b) due to elevated activity of catalase (CAT) (Fig. 3, e, f), reduced peroxidase (PO) (see Fig. 3, c, d) and oxalate oxidase (OXO) (see Fig. 3, c, d) activities, and lack of transcript accumulation of the genes encoding oxidoreductases NADPH oxidase (TaRbohF), SOD (TaSod), and PO (TaPrx) (Table 3). However, in Tox3-insensitive plants (Om35/SnB+ET cultivar/isolate combination), the suppression reaction of oxidative burst was less pronounced than in other combinations leading to a compatibility reaction (see Fig. 2, 3, Table 3).
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Fig. 3. Enzyme activities in leaves 24 and 72 h after inoculation with S. nodorum SnB (Tox3+) and Sn4VD (Tox3–) isolates: (a, b) peroxidase; (c, d) oxalate oxidase; (e, f) catalase; (a, c, e) cv. Kazakhstanskaya 10; (b, d, f) Omskaya 35.

Designations follow Fig. 2.

Table 3. The results of transcriptional analysis of oxidoreductase genes registered 24 h after inoculation with S. nodorum isolates in wheat cultivars Kazakhstanskaya 10 and Omskaya 35 characterized by different levels of resistance to the disease

| Variant of treatment* | Pathogen isolate |
|----------------------|------------------|
|                      | Kazakhstanskaya 10 | Omskaya 35 |
|                      | SnB (Tox3+)       | Sn4VD (Tox3–) |
| TaRboh               | TaSod             | TaPrx       |
| Control              | 100               | 100         | 100         | 100         | 100         |
| Sn                   | 78 ± 3            | 88 ± 10     | 71 ± 3      | 160 ± 21    | 144 ± 8     |
|                      | 223 ± 20          | 160 ± 21    | 144 ± 8     | 223 ± 20    |
| Sn + AVG             | 111 ± 20          | 169 ± 20    | 163 ± 30    | 145 ± 40    | 110 ± 3     |
|                      | 210 ± 50          | 145 ± 40    | 110 ± 3     | 210 ± 50    |
| Sn + ET              | 15 ± 1            | 81 ± 7      | 104 ± 20    | 152 ± 37    | 114 ± 2     |
|                      | 251 ± 50          | 152 ± 37    | 114 ± 2     | 251 ± 50    |

| Control              | 100               | 100         | 100         | 100         | 100         |
| Sn                   | 126 ± 10          | 264 ± 20    | 365 ± 26    | 160 ± 20    | 196 ± 9     |
|                      | 264 ± 20          | 365 ± 26    | 160 ± 20    | 196 ± 9     |
| Sn + AVG             | 136 ± 20          | 242 ± 40    | 311 ± 31    | 129 ± 10    | 178 ± 18    |
|                      | 170 ± 20          | 311 ± 31    | 129 ± 10    | 178 ± 18    |
| Sn + ET              | 83 ± 10           | 118 ± 9     | 136 ± 15    | 132 ± 30    | 159 ± 10    |
|                      | 181 ± 34          | 136 ± 15    | 132 ± 30    | 159 ± 10    |

* Treatment options are designated as in Fig. 2.
of pathogen growth (see Table 1). Treatment with ET did not affect the nature of the response to inoculation with the Sn4VD isolate in either Tox3-sensitive or insensitive plants (see Fig. 2, 3, Table 3).

Discussion

At present, it is known that S. nodorum produces eight NEs associated with the virulence of pathogen isolates (Phan et al., 2016; Shi et al., 2016). The main NEs are SnToxA, SnTox1, SnTox3 toxins, which are considered to be the key factors of the virulence of pathogen strains and isolates, as shown in experiments with mutant S. nodorum strains and different wheat lines sensitive and insensitive to these effectors (Phan et al., 2016; Shi et al., 2016).

In our study, high transcriptional activity of the SnTox3 gene was found in the virulent isolate SnB (Fig. 1, b), which may be indicative of SnTox3 inactivation in this isolate (Tan, Oliver, 2017). Here we studied two cultivars of soft spring wheat in resistance to S. nodorum and differing in the allelic composition of the Snn3-B1 locus (see Fig. 1, c). This difference is presumed to be associated with their sensitivity and insensitivity to NE SnTox3 (Shi et al., 2016). In the referred study, two BG220 and Sumai3 wheat lines carrying different alleles of the Snn3-B1 locus showed different degrees of sensitivity to NE SnTox3. Despite the fact that the Snn3-B1 null allele was not detected in cv. Om35, this cultivar was insensitive or weakly sensitive to SnTox3. This may indicate a large deletion in the locus between the Xcfd20 and Xgwm234 markers, which is in agreement with the literature data (Shi et al., 2016). Thus, out of 17 Sumai3 mutants insensitive to SnTox3, the Snn3-B1 null allele was detected only in 5 of them: two lines harbored null alleles for three microsatellite markers, Xgwm234, Xmag705 and Xcjb306, and three lines had a null allele for one microsatellite marker Xcjb306 (Shi et al., 2016). Thus, four different cultivar/isolate combinations were selected with two isolates of S. nodorum SnB (Tox3+) and Sn4VD (Tox3-) and two cultivars of spring common wheat with different genotypes to study the role of SnTox3 in the development of infection.

On the one hand, the main function of NE SnTox3 is the formation of lesion zones on the wheat leaves of sensitive genotypes by hijacking host’s nonspecific signaling defense pathways and manipulating them for pathogen growth and propagation (Winterberg et al., 2014). Furthermore, SnTox3 has been shown to increase ethylene synthesis in infected plants (Winterberg et al., 2014).

To elucidate the role of SnTox3 in ethylene biosynthesis and signaling pathways, we treated part of wheat plants of two cultivars, Kaz10 and Om35, with ethylene chemical precursor ET and ethylene biosynthesis inhibitor AVG. The results showed that both the elevated susceptibility of the ET-treated plants to S. nodorum and elevated resistance of the AVG-treated plants depended on the pathogen genotype: Tox3+ in SnB or Tox3- in Sn4VD (see Table 1). This observation suggests that NE SnTox3 acts as a virulence factor and affects the plant defensive system by regulating ethylene biosynthesis and signaling pathways. It has been shown that ethylene production by some pathogens is closely associated with their virulence (Ma K.-W., Ma W., 2016). For instance, the XopD effector of the pathogenic bacterium Xanthomonas euvesicatoria manipulates the ethylene signaling pathway, affecting transcription factor ERF4 (Ma K.-W., Ma W., 2016), and the necrotrophic fungus Cochliobolus miyabeanus induces the ethylene signaling pathway in rice to produce and secrete ethylene as an effecter to accelerate infection (Shen et al., 2018).

In our work, the analysis of the transcriptional activity of the genes controlling the biosynthesis and signaling pathway of ethylene also showed that the activation of genes involved in this pathway in infected plants depended on the pathogen isolate genotype and the sensitivity of the wheat genotype to NE SnTox3 (see Table 2). These results suggest that the biosynthesis and signaling pathway of ethylene are induced in the gene-for-gene interaction between Snn3-B1 and SnTox3.

Interestingly, in SnTox3 insensitive plants (Om35/SnB+ET cultivar/isolate combination), ET treatment increased plant sensitivity to this NE (see Table 3), suggesting that such a reaction could result from a mutation in genes regulated by the Snn3-B1-SnTox3 interaction (Shi et al., 2016). However, the activation of genes for ethylene biosynthesis and the signaling pathway in the Om35/SnB+ET combination was weaker than in case of the compatible interaction in plants sensitive to SnTox3 (Kaz10/SnB, Kaz10/SnB+ET) (see Table 2). This suggests that resistant plants possess a mechanism for efficient suppression of ethylene biosynthesis and signaling pathway to induce defense responses that are inhibited by ethylene. For example, ethylene inhibited salicylic acid (SA) biosynthesis and suppressed the expression of the SA-mediated signaling pathway marker genes PR-1 and PR-2 in Arabidopsis plants infected with Pseudomonas syringae (Chen et al., 2009), as well as in wheat plants infected with S. nodorum (Veselova et al., 2016). Silicon blocked ethylene production by the pathogen C. miyabeanus, which improved the resistance of rice plants (Shen et al., 2018). Thus, the obtained results prove the influence of SnTox3 on the biosynthesis and signaling pathway of ethylene in the course of Snn3-B1-SnTox3 interaction according to the gene-for-gene type with ultimate suppression of the defense reactions of wheat plants to facilitate colonization.

Our previous studies showed that ethylene provided comfortable conditions for the penetration and development of S. nodorum in wheat plant tissues at the initial stage of infection due to the regulation of redox metabolism and reduction of H2O2 generation (Veselova et al., 2016, 2018). On the contrary, the accumulation of ROS in wheat plants at the initial stage of infection with the pathogen S. nodorum determined the resistance of the cultivar, inducing the expression of the genes encoding pathogenicity-related proteins (Veselova et al., 2016, 2018). The change in the redox state of infected wheat plants in our experiments completely depended on the activation or inhibition of the biosynthesis and signaling pathway of ethylene, and this effect was due to the Snn3-B1-SnTox3 interaction of the gene-for-gene type (see Fig. 2, 3). Our results demonstrate that ethylene suppresses H2O2 accumulation in plants sensitive to SnTox3 via increasing CAT activity, reducing PO and OXO activities, and lowering the transcript contents of genes encoding NADPH oxidase and SOD, in consistency with literature data (Golemiec et al., 2014; Ma et al., 2017) and our earlier results (Veselova et al., 2018).
Formerly, it was shown that NE SnTox3 regulated genes involved in redox metabolism and the formation of necrosis (Winterberg et al., 2014), but the mechanisms effecting the influence of SnTox3 on ROS generation remain obscure. It is known from the literature that specific effectors of pathogens can induce hypersensitive response in plant cells and suppress the oxidative burst in plants during the infection process in various ways (Jwa, Hwang, 2017). For example, two cytoplasmic effectors of Phytophthora sojae interact with catalases to regulate H$_2$O$_2$ concentration. The Pep1 effector of U. maydis interacts with POX12 maize peroxidase in vivo and suppresses early immune responses in maize (Hemetsberger et al., 2012).

Conclusion

The results of our work suggest that the pathogen effector SnTox3 influences biosynthesis and signaling pathway of ethylene in order to regulate the redox metabolism of infected wheat plants in the way promoting successful colonization of the host at initial stages of infection, which subsequently gives rise to extensive damage lesions due to fast pathogen reproduction.

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Acknowledgements. This work was supported by State Project AAAA-A16-116020350027-7 and the Russian Foundation for Basic Research, project 18-04-00978. The authors are grateful to the staffs of the “Biomika” Shared Access Center (Branch of Biochemical Methods and Nanobiotechnology, “Agidel” Resource Center for Collective Use) and the “KODINK” Complex of Equipment for the Study of Nucleic Acids for access to the equipment.

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

Received June 1, 2019. Revised July 8, 2019. Accepted July 22, 2019.